Myocardial Perfusion and Resistive Vessel Function in Coronary Artery Disease

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1993

For Janet, my wife,
and William, my son.

DECLARATION OF THESIS

I declare that this entire body of work has been composed and written by me and that the work reported herein is my own. This work was done whilst I was in post at the Royal Postgraduate Medical School, Hammersmith Hospital, London, as a British Heart Foundation Junior Research Fellow and I acknowledge the financial support of the British Heart Foundation over these two years. I have organised, collected and analysed all the data reported in this thesis. A proportion of this work involved invasive study of patients undergoing clinical investigation in the cardiac catheterisation laboratory. In this respect, I acknowledge the help of Drs. Tom Crake, David Lefroy and Grahan Davies with regard to data derived from coronary arteriography and to Drs. Paolo Marraccini, Roberto Gistri and Paolo Camici in Pias with regard to data derived from coronary sinus catheterisation in a selected group of patients. I can confirm that I have not submitted the thesis in candidature for any other degree, diploma or professional qualification.

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Signed the 24th Day of June, 1993

UNIVERSITY OF EDINBURGH

ABSTRACT OF THESIS (Regulation 3.5.10)

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Myocardial blood flow (MBF) is regulated by the coronary resistive vessels which continuously adapt the coronary blood flow the myocardial metabolic requirements, modulated by neural and humoral mechanisms, and this adaptation can compensate for increased resistance of an epicardial stenosis to a considerable extent. In patients with coronary artery disease, we postulate t dysfunction of the coronary resistive vessels may cause or contribute to myocardial ischaemia. Thus, impaired myocardial rfusion maybe due to the abnormal behaviour of collateral and resistive vessels rather than to epicardial disease alone. We pose that this alteration in resistive vessel function occurs, not only in regions subtended by epicardial disease, but is present in note myocardium and may be altered by coronary intervention such as coronary angioplasty (PTCA) and after myocardial arction (MI). To investigate coronary resistive vessel function, positron emission tomography (PET) may be used to evaluate ional MBF using the flow tracer ^{15}O-labelled water. Using vasodilator (or vasoconstrictor) stimuli, the coronary vasodilator ponse (CVR=maximal/basal coronary [myocardial] blood flow), an index of coronary resistive vessel function, may be measured d compared in regions of interest and in remote myocardium.

Recovery of Resistive Vessel Dysfunction After Successful PTCA. To investigate the frequency and the time course of abnormal onary resistive vessel function after successful PTCA, patients with single vessel coronary disease and normal left ventricular ction underwent intracoronary (IC) Doppler measurement of coronary flow velocity, before and after successful PTCA, at basal d after intravenous (IV) dipyridamole. PET was performed on 3 occasions after PTCA. There was no change in CVR at Doppler er PTCA. In patients without restenosis, the CVR was reduced in the PTCA region for ≥7 days, but returned to normal at 3 nths, due to increased basal MBF for ≥7 days in the PTCA region, with a reduction in the dipyridamole-induced maximal MBF ≥ 24 hours.

Altered Nitric Oxide Synthesis/Release and Resistive Vessel Dysfunction After PTCA. Impaired production or release of nitric de (NO) in the from resistive vessel endothelium may cause this alteration in the CVR after PTCA. As the CVR to exogenous rates is enhanced by the endothelial dysfunction, large doses of IC sodium nitroprusside, an NO donor, were infused at the peak ect of IV dipyridamole to test this hypothesis using Doppler catheterisation in patients with single vessel disease. roprusside in doses sufficient to cause ultimately a fall in blood pressure did not augment the dipyridamole-induced increase in onary blood flow velocity.

Altered Flow and Metabolism in Regions Subtended by Angiographically Normal Arteries in Coronary Artery Disease. The ional CVR was measured using PET in patients with stable single vessel disease. In a second group of patients and controls, ultaneous arterial and great cardiac vein catheterisation was done at rest and during atrial pacing to measure myocardial tabolism in regions subtended by a diseased artery or by an angiographically normal artery with epicardial disease elsewhere. e CVR was reduced in remote regions compared to controls. In the second group, at maximal pacing, there was net lactate duction in the diseased region compared to net extraction in both the remote and control regions. However, glucose and alanine raction were increased in both diseased region and remote regions compared to control.

Resistive Vessel Dysfunction in Infarcted and Remote Myocardium After MI. To investigate acute resistive vessel dysfunction, ients were studied after thrombolysis for MI. Regional MBF and the CVR in infarct and remote regions was assessed, after a an of 8 days and 6 months after MI by PET. At early scanning, the CVR was markedly reduced in the infarct region, and was ated to the amount of residual viable tissue. There was no correlation between the CVR and residual stenosis area. The remote R was less than that in remote regions, subtended by a normal artery, in controls with stable single vessel disease without MI. late scanning, the CVR improved in the infarct region, but the CVR in the remote region still remained lower than in controls.

Impaired Flow Response to Cold Pressor in Collateral-Dependent Myocardium. To investigate the response of collateral-pendent myocardium to reflex sympathetic stimulation (cold pressor stress), patients with stable angina and normal left tricular function were studied, in whom one coronary artery was occluded (without previous MI), and the other arteries were giographically normal supplying collaterals. Regional MBF and glucose uptake (using ^{18}F-deoxyglucose) was measured using T at basal and at cold pressor. With cold pressor, no patients developed ECG changes. The cold pressor response (cold ssor/basal MBF) was low in the collateralised region, compared to remote regions, due to vasoconstriction in the majority, but in absence of demonstrable ischaemia.

In summary, there is coronary resistive vessel dysfunction after PTCA which recovers over 3 months due to acute impairment of response to dipyridamole and a longer increase in basal flow, possibly due to the previous stenosis. This impairment is not due altered production or release of NO in the microcirculation. In stable disease, there is both an impaired CVR and altered tabolism during pacing in regions subtended by a normal artery. This remote alteration is impaired acutely by myocardial arction elsewhere, with only incomplete recovery over at least 6 months. In addition to reduced vasodilator function, resistive sels in patients with atherosclerosis, have an increased tendency to vasoconstrict to a sympathetic stimulus. Thus, the erosclerotic process and the sympathetic nervous system may both play a role in determining the degree of resistive vessel function, which may cause or contribute to myocardial ischaemia in patients with coronary artery disease.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AA	=	arachidonic acid
ACh	=	acetylcholine
ADP	=	adenosine diphosphate
ATP	=	adenosine triphosphate
cAMP	=	cyclic adenosine 3',5'-monophosphate
$C^{15}O$	=	15-oxygen-labelled carbon monoxide
$C^{15}O_2$	=	15-oxygen-labelled carbon dioxide
ECG	=	electrocardiogram/electrocardiographic
EDCF	=	endothelium-derived contracting factor
EDRF	=	endothelium-derived relaxing factor
EDHF	=	endothelium-derived hyperpolarising factor
FDG	=	[^{18}F] 2-fluoro 2-deoxyglucose
5-HT	=	5-hydroxytryptamine (serotonin)
FWHM	=	full width at half maximum
cGMP	=	cyclic guanosine 3',5'-monophosphate
H^+	=	hydrogen ions
$H^{15}O_2$	=	15-oxygen-labelled water
LAD	=	left anterior descending artery
LCX	=	left circumflex artery
LSF	=	line spread function
LV	=	left ventricular
MAO	=	monoamine oxidase
MBF	=	myocardial blood flow
$^{13}NH_3$	=	13-nitrogen-labelled ammonia
NAD	=	nicotinamide dinucleotide
NADH	=	nicotinamide dinucleotide (reduced form)
PET	=	positron emission tomography
PGI_2	=	prostacyclin
Pi	=	inorganic phosphate
pO_2	=	partial pressure of oxygen
PTCA	=	percutaneous transluminal coronary angioplasty
RCA	=	right coronary artery
^{82}Rb	=	82-rubidium
TXA_2	=	thromboxane A_2

CHAPTER 1. CORONARY RESISTIVE VESSEL FUNCTION AND THE CONTROL OF THE CORONARY CIRCULATION

1.1 Introduction

In the normal heart, myocardial blood flow is regulated by the coronary resistive vessels which continuously adapt the coronary blood flow to the myocardial metabolic requirements. This local metabolic autoregulatory control is also modulated by neural and by humoral mechanisms. In the presence of an epicardial coronary artery stenosis, this mechanism can compensate for the resistance created by the stenosis to a considerable extent, as animal studies show that myocardial blood flow can still increase 3-4 times above resting levels even distal to a 70% diameter stenosis (Gould et al, 1975).

In patients with coronary artery disease, recent observations suggest that the behaviour of the resistive vessels is abnormal and may contribute to the development of myocardial ischaemia. However, in the presence of a coronary artery stenosis, it is impossible to separate modulation of coronary blood flow at the level of the stenosis (Epstein & Talbot, 1981), from that occurring in the resistive vessels. Therefore, in order to obviate the difficulty in establishing whether or not the modulation of coronary blood flow is caused by changes in the calibre of the coronary stenosis (Wilson et al, 1987), we propose to assess the modulation of coronary blood flow in models of disease which would allow us to exclude this possibility. Furthermore, it is possible that resistive vessel dysfunction may occur as part of the generalised process of atherosclerosis or through local changes in autoregulation or autonomic modulation, and may occur in coronary vascular beds subtended by angiographically normal epicardial arteries. Thus, it is important when observing resistive vessel function that

simultaneous assessment is made in such regions when comparing with regions subtended by epicardial arteries with obvious disease.

The function of the human coronary resistive vessels in vivo can only be assessed by measurement of myocardial blood flow. Whether or not resistive vessel dysfunction leads to recurrent myocardial ischaemia is more difficult to ascertain. In the presence of abnormal resistive vessel function, ischaemia must be assessed directly because, as in syndrome X which may be characterised by dysfunction of the coronary resistive vessels (Cannon et al, 1992), ischaemic electrocardiographic (ECG) changes may not be associated with direct evidence of myocardial ischaemia (Crake et al, 1988).

1.2 Coronary Blood Flow

Myocardial performance is determined by coronary blood flow which is dependent on the coronary perfusion pressure and the coronary vascular resistance. Unlike other vascular beds, the resistance of the coronary circulation is influenced by the phasic systolic compression of the coronary vessels within the myocardium. The effective coronary perfusion pressure is the pressure gradient between the large coronary arteries and the right atrium or left ventricle in end-diastole, which change with the cardiac cycle. Coronary vascular resistance is influenced both by extrinsic factors such as myocardial compression and by intrinsic factors such as tissue metabolism, and neural and humoral influences. Although coronary flow occurs in both systole and diastole, this is mainly true only of the right coronary artery, as flow is predominately diastolic in the left coronary circulation. The calculation of coronary vascular resistance is complicated by the fact that coronary blood flow ceases when the coronary perfusion pressure falls to approximately 40 mmHg (pressure at zero flow), still substantially above coronary venous pressure (Klocke et al, 1985). Although the

exact mechanism for this is unclear, intra-myocardial pressure accounts for most of this pressure.

With the penetration of the myocardium from the epicardial surface, smaller coronary arteries are exposed to progressively greater systolic pressures, with greatest extravascular resistance to flow in the subendocardial myocardial layers. Because of its increased dependence on diastolic perfusion and the greater degree of systolic shortening (and thus of energy expenditure), the subendocardium has an increased susceptibility to the development of myocardial ischaemia (Bell & Fox, 1974). Under normal circumstances, secondary to this increased wall stress and thus increased myocardial oxygen consumption, but lower perfusion pressure, in the subendocardium, there is preferential subendocardial vasodilatation with flow 25% greater than in the subepicardium (Klocke, 1976). This means that the coronary vasodilator reserve (Chapter 2, Section 2.3) is lower in the subendocardium and that it is more susceptible to myocardial ischaemia when there is an increase in myocardial oxygen demand or a decrease in myocardial oxygen supply such as seen with a vasodilator stimulus in the presence of an epicardial stenosis. This is because resistive vessel dilatation leads to a reduction in perfusion pressure as the stenosis prevents an increase in flow in response (Bache & Schwartz, 1982). A reduced intravascular distending pressure and increased extravascular compression redistribute flow away from the subendocardium, leading to ischaemia.

1.3 The Coronary Resistive Vessels

The coronary circulation has been traditionally divided into two arterial compartments; the large epicardial conduit vessels and the small resistance (resistive) vessels, and one venous compartment, which regulate the flow of blood. It was previously believed that the control of coronary resistance resided entirely in this

homogeneous compartment of resistive vessels throughout the myocardium, modified in normal subjects by the coronary perfusion pressure, myocardial oxygen demand, ventricular wall tension and the metabolic state of the coronary vascular bed. In recent times, it has become apparent that there is substantial heterogeneity of function in each of the vascular compartments, but particularly with respect to the regulation of coronary flow by the resistive vessels.

Resistive vessels have two major functions; i) to match myocardial blood flow to oxygen consumption when myocardial demand varies, and ii) to match myocardial blood flow to myocardial demand when aortic pressure (coronary perfusion pressure) varies. Resistive vessels function to continually match blood supply to metabolic requirements such that myocardial oxygen extraction is virtually constant over a wide range of cardiac work and coronary perfusion pressure.

Several techniques have been developed over the years to study the coronary microcirculation with direct visualisation. These methods have employed an animal model using regional immobilisation and microscopic examination (Tillmanns et al, 1981). With the development of a stroboscopic method (Nellis et al, 1981) has come additional refinements requiring limited restriction of cardiac movement, although confined to study of only the microvessels closest to the surface of the heart (Marcus et al, 1990). Techniques have also been developed to study vessels in vitro and it is now possible to visualize vessels as small as 15 μm in the beating heart (Kuo et al, 1988). From these studies, much of what is known about resistive vessel function has been discovered, although it is important to add the caveat that these studies have been done in an artificial environment free from the physiological constraints of normal cardiac metabolism and varying myocardial oxygen consumption.

1.3.1. Distribution of the Coronary Resistance/

1.3.1. Distribution of the Coronary Resistance

Conventional teaching believed that most of the resistance to coronary flow resided in arterioles smaller than 100 μm (Zweifach et al, 1984). However, it has become apparent that a significant proportion of resistance is present in larger arterioles. In the cat, 50% of coronary resistance is in vessels >100 μm (Chilian et al, 1986a), and 25% of coronary resistance is in vessels >170 μm under basal conditions (Chilian et al, 1989a). As 10% of resistance is in the venous compartment, this leaves about 40% of resistance in the pre-capillary resistive vessels <100 μm. Pharmacological vasodilatation can shift the distribution of resistance from one compartment to another; dipyridamole (and adenosine) reduces resistance in resistive vessels <150-170 μm (Chilian et al, 1989a; Kanatsuka et al, 1989a). This causes an increased proportion of total resistance to move to the venous compartment and larger arteries (Chilian et al, 1989a) (Figure 1.1), implying that flow-mediated vasodilatation in the larger resistive vessels was less than required to fully compensate for the increase in flow. By contrast, papaverine preferentially vasodilates larger coronary arterioles and small coronary arteries (>200 μm) (Chilian et al, 1986a). The concept that coronary resistance coexists in different arteriolar compartments and that the relationship may change depending on the perfusion pressure and local metabolic environment is of importance in understanding the control of coronary flow (Section 1.4.1.4). That this relationship may be disturbed in patients with coronary artery disease is central to the working hypothesis of this thesis.

1.4 The Control of Myocardial Perfusion

Myocardial perfusion is controlled by the modulation of coronary flow to perfusion pressure - autoregulation - and by tissue metabolism. Coronary autoregulation is the intrinsic ability of the heart to maintain myocardial blood flow

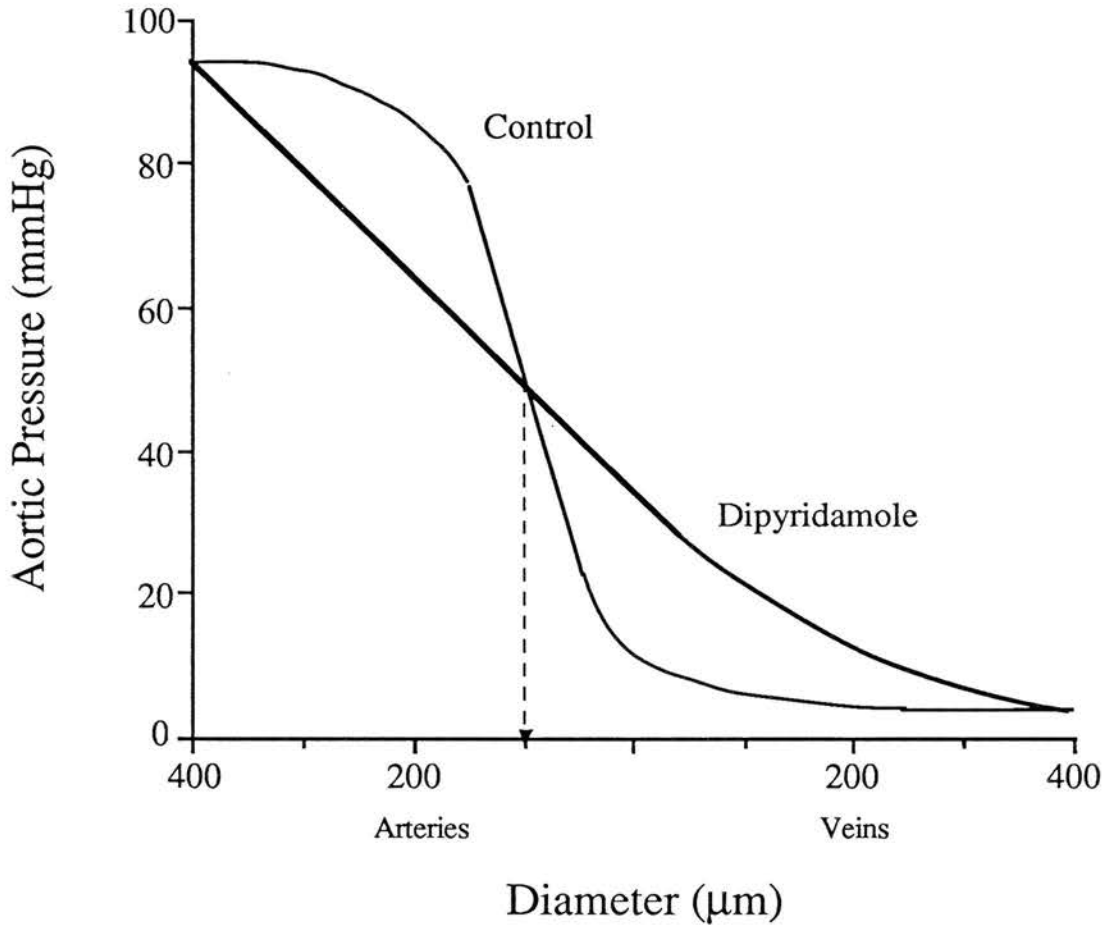


Figure 1.1. Distribution of microvascular pressure measured using the servonull technique in relation to aortic pressure in different sized coronary arterioles and venules in the normal left ventricle in pentobarbitol anaesthetized cats during control conditions (thin line) and intravenous dipyridamole at 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$ (thick line). During infusion of dipyridamole, there is a redistribution of microvascular resistance such that larger arteries and veins account for a greater portion of resistance. The dashed line identifies 100 μm diameter arterioles (Modified from Chilian et al, 1989a).

relatively constant following changes in perfusion pressure when myocardial oxygen demands are constant (Dole, 1987). This a local autonomous form of control which may be influenced by extrinsic factors such as autonomic nervous system activity and circulating hormones. To study autoregulation, it is important to separate the effects of aortic pressure from coronary perfusion pressure since changes in the former, a determinant of myocardial oxygen demand, may lead to changes in myocardial tissue metabolism (the other major determinant of myocardial perfusion). Autoregulation can be said to occur where the relative change in flow is proportionally less than a change in perfusion pressure, such that vascular resistance changes in the same direction as pressure, whether acutely or as a chronic adaptation, to return flow to or close to basal steady state values (Mosher et al, 1964). In the coronary circulation, this generally occurs between perfusion pressures of 60 and 140 mmHg (Klocke, 1987) (Figure 1.2).

Autoregulation is an important homeostatic mechanism for maintaining nutritive flow in the face of a reduction in perfusion pressure whether caused by systemic hypotension or a proximal flow obstruction. Conventionally, it may be demonstrated by measuring coronary flow over a range of coronary perfusion pressures. At a perfusion pressure of 60 mmHg coronary flow is maintained because of vasodilatation of the resistive vessels <100 μm diameter and the magnitude of vasodilatation is inversely proportional to the vessel diameter. At a perfusion pressure of 40 mmHg which is below the autoregulatory range, although these vessels <100 μm diameter dilate towards their physiological maximum, vessels >100 μm decrease in diameter (Kanatsuka et al, 1989b). This reduction in diameter is passive to an extent as these vessels are still amenable to vasodilatation with nitroglycerin and adenosine. This may be evidence for an abnormality in these resistive vessels >100 μm (the so-called pre-arteriolar vessels) occurring in the equivalent, albeit more chronic, setting of a proximal flow obstruction. These myogenic responses (Section 1.4.1.2) are less

apparent in subendocardial vessels: perhaps another reason for the reduced efficacy of autoregulation in the subendocardium (Section 1.2). An increase in myocardial oxygen demand with pacing leading to an increase in tissue metabolism leads to a generalised vasodilatation in all resistive vessels, although again related inversely to initial diameter (Kanatsuka et al, 1989a). This may be because the arterioles (<100 μm) are subject to a lower transmural pressure (Nellis et al, 1981; Tillmanns et al, 1981), but also, these arterioles are only resistive vessels influenced by the tissue metabolic state (Section 1.4.1.4). Thus, the response of the resistive vessels to different physiological stimuli is heterogeneous.

It is likely that several different mechanisms may account for the heterogeneity of the vasodilator response of the resistive vessels. Comparing large and small arterioles, there may be differential receptor populations with different receptor subtypes for vasoactive substances released in the coronary microcirculation such as 5-HT_1 and 5-HT_2 receptors for serotonin (Lamping et al, 1989). It is also possible that endothelium-derived relaxing factor (EDRF; nitric oxide) is released to a varying degree in differently sized vessels (Kurz et al, 1989) or that differently sized vessels respond according to the immediate coronary flow at the origin of that vascular bed, dependent on the vasodilated/vasoconstricted state of the upstream resistive vessels. To some extent, this may also account for the differential roles these vessels have in the autoregulation of blood flow (Section 1.4.1.4).

The reasons for the apparent submaximal dilatation of the resistive vessels when perfusion pressure is reduced below autoregulatory range (Canty et al, 1985), manifest by residual dilatation to adenosine for example (Grattan et al, 1986; Chilian & Layne, 1990), are not known. There would seem to be a difference between the ability to vasodilate endogenously and to vasodilate to a pharmacological stimulus. This may be due to a reduction in the production of vasodilator metabolites through reduced myocardial oxygen consumption which in clinical terms would limit

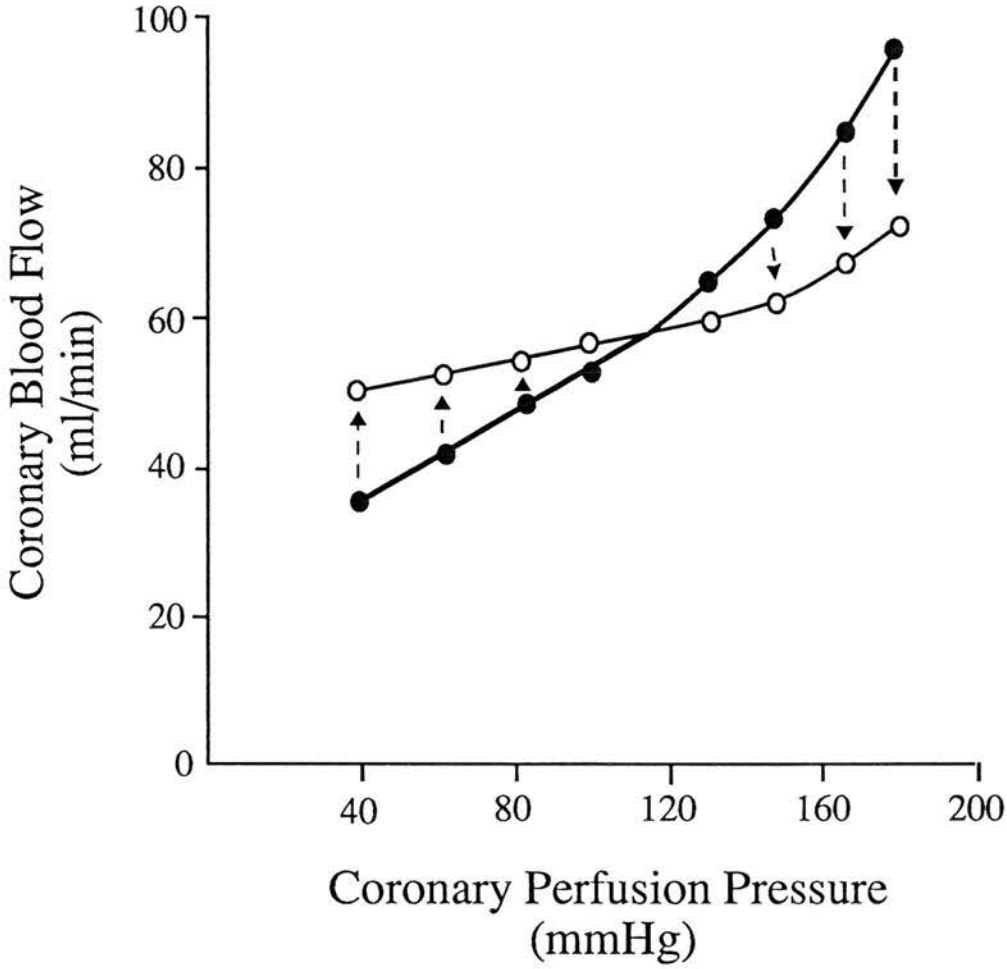


Figure 1.2. Autoregulation of coronary blood flow in the beating dog heart. The point where the curves cross represents control steady-state pressure and flow. A sudden sustained change in perfusion pressure leads to an abrupt change in flow represented by the closed symbols and the thick line (transient flow). The open symbols and thin line represent steady-state flows obtained at each perfusion pressure. (Modified from Rubio & Berne, 1975).

irreversible ischaemic dysfunction, the experimental equivalent of myocardial stunning. Alternatively, it is possible that pharmacological vasodilatation overcomes a strong endogenous vasoconstrictor component due to metabolites such as prostaglandins or leucotrienes (Aversano & Becker, 1985) or through post-junctional α_2-adrenergic mediated vasoconstriction (Heusch & Deussen, 1983) (Section 1.4.2.1).

1.4.1. Mechanisms of Coronary Autoregulation

1.4.1.1. The Tissue Pressure Theory

This hypothesis postulates that autoregulation occurs through passive changes in extravascular compression of compliant vessels due to increased or decreased capillary filtration in response to changes in perfusion pressure. However, it is unlikely that such a mechanism could account for rapid dynamic changes in perfusion pressure (Driscol et al, 1963). Furthermore, using a micropipette system to measure intramyocardial pressure in the beating dog heart, it has been shown that changes in aortic pressure, and thus perfusion pressure, did not affect diastolic tissue pressure (Heineman & Grayson, 1985).

1.4.1.2. The Myogenic Theory

This hypothesis proposes that vascular resistance is proportional to transmural pressure in the microcirculation, through the effect of pressure on vascular smooth muscle (Folkow, 1964). This mechanism does contribute to autoregulation in vascular beds of other organs, and although the evidence from the coronary vascular bed is less firm, adaptive vasomotor changes do occur through alterations in myogenic tone in concert with intravascular distending pressure and tissue metabolism (McHale et al, 1987). The argument that myogenic regulation might occur was based on the assumption that brief periods of occlusion were insufficient to alter tissue metabolism.

However, it has been shown by ^{31}P-magnetic resonance spectroscopy that the metabolic response of the heart to changes in flow is very rapid (Fossel et al, 1980). In addition, by reducing myocardial oxygen demand through lowering heart rate, it is possible to attenuate autoregulation, again against a myogenic mechanism (Dole & Nuno, 1986).

1.4.1.3. The Metabolic Theory

This hypothesis proposes that tissue metabolism feeds back through the production of vasoactive metabolites to control coronary vascular resistance. A major problem in distinguishing metabolic and myogenic mechanisms of autoregulation has been because changes in coronary perfusion pressure (the myogenic stimulus) result directly in similar changes in blood flow (the metabolic stimulus). Indeed, when myocardial oxygen consumption is constant, coronary flow autoregulation may still occur. Much research into the metabolic control of autoregulation has been to observe flow during interventions which alter the myocardial oxygen demand. Reducing demand may attenuate autoregulation completely or may simply move the pressure-flow relationship down parallel to basal conditions. This variability could be due to the effects of any given intervention on myocardial oxygen tension, as it has been demonstrated that the degree of autoregulation may depend largely on the balance between myocardial oxygen supply and demand rather than on the absolute level of myocardial oxygen consumption (Dole & Nuno, 1986).

Coronary autoregulation appears to be closely related to myocardial oxidative metabolism. It is likely that no one substrate, such as adenosine, or metabolite accounts for this and that autoregulation results from the concerted interaction of several different mediators, such that one may compensate for another should it be reduced or blocked by pharmacological means or myocardial ischaemia.

1.4.1.4. The Pre-arteriolar/Arteriolar Theory

All the above theories on autoregulation of myocardial blood flow rely on an over-simplistic and unitary understanding of the coronary resistive vessels, despite much recent evidence for distinct functional heterogeneity of these vessels (Marcus et al, 1990). It is likely that there is both a myogenic and a metabolic component to the control of regional transmural coronary microvascular flow and that under normal conditions, these mechanisms act to complement each other.

There is existing evidence to show that myocardial ischaemia may be caused by inappropriate vasoconstriction of resistive vessels (Wilson et al, 1988; Pupita et al, 1990; Marcus et al, 1990) and the vessels involved in such vasoconstriction may not necessarily the same as those involved in metabolically-mediated vasoconstriction. As described above, reduction of the coronary perfusion pressure below the autoregulatory range dilates vessels <100 μm diameter but ultimately leads to constriction of the larger resistive vessels with an increasingly severe stenosis (Kanatsuka et al, 1989b). This constriction could occur passively and reflect the reduced distending pressure and myogenic tone, but also account for their residual response to pharmacological vasodilatation (Berne & Rubio, 1979). An alternative explanation is the presence of α-adrenergic vasoconstriction which occurs with the reduction of perfusion pressure (Feigl et al, 1987) (Section 1.4.2.1). Sympathetic nerve stimulation or noradrenaline infusion (Chilian et al, 1989b) and serotonin infusion (Lamping et al, 1989) constrict vessels >90-100 μm, dilating smaller vessels. This may be evidence for the compensatory response of metabolic vasodilatation to counter pre-arteriolar constriction to allow maintenance of perfusion pressure.

On theoretical grounds, one can separate these resistive vessels into two groups on the basis of function and diameter (Epstein & Cannon, 1986). These are the distal "arteriolar" vessels (<100 μm) - predominately responsive to local tissue metabolism and which directly match supply to demand (Kanatsuka et al, 1989a). This occurs to

maintain the intracellular environment with optimal biochemical limits for myocardial contractile function and is modulated primarily by tissue oxygen and carbon dioxide tension (Hoffman & Spaan, 1990). The flow in the arterioles is dependent on their inherent tone and the perfusion pressure at origin. This is determined by the second group - the proximal "pre-arteriolar" vessels (100-350 μm) - influenced only by coronary perfusion pressure, and thus coronary flow and distending pressure, and by myogenic tone (Maseri et al, 1991). Although there is no obvious histological demarcation between these two groups, it is likely that the pre-arteriolar vessels have a supply of autonomic afferent nerves which may serve to modulate inherent myogenic tone. When there is an increase in myocardial oxygen demand with arteriolar vasodilatation, a pressure drop would potentially occur across the pre-arteriolar vessels with increasing flow. Flow-mediated vasodilatation at this level allows maintenance of increased flow sufficient to meet tissue needs. With an increase in aortic pressure, arteriolar pressure may be maintained by pre-arteriolar constriction through an increase in myogenic tone. A marked decrease in aortic pressure leads to dilatation of all resistive vessels (Kanatsuka et al, 1989a). In pathological conditions, a failure of pre-arteriolar vessels to dilate or to constrict inappropriately would lead to a reduction in flow in the arteriolar bed despite maximal dilatation in response to tissue metabolism (Figure 1.3).

This hypothesis of the regulation of myocardial perfusion at a microvascular level may be viewed to encompass both the metabolic theory and myogenic theory described above.

i) *Regulation of myocardial blood flow when aortic pressure changes*

As described above, the process of autoregulation allows very little change in myocardial blood flow over a wide range of perfusion pressures. The regulatory mechanisms may change as perfusion pressure rises above or falls below this

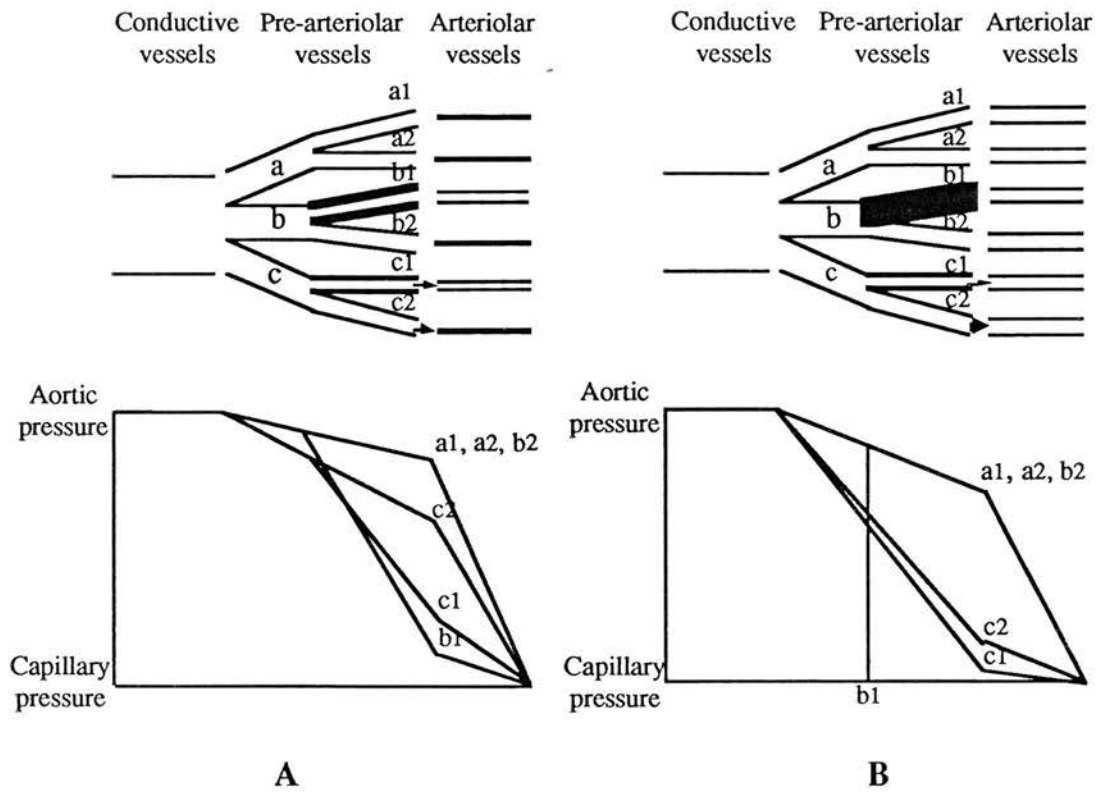


Figure 1.3. Schematic representation of a conduit (conductive) artery and pre-arteriolar and arteriolar vessels with patchily distributed pre-arteriolar constriction in control conditions **(A, upper panel)** and during arteriolar vasodilatation **(B, upper panel)**. In this functional classification, conduit coronary arteries do not have appreciable flow resistance, arteriolar vessels are responsible for the metabolic autoregulation of coronary blood flow and pre-arteriolar vessels are those segments interposed between conductive and arteriolar vessels, with appreciable coronary flow resistance that are responsible for maintaining perfusion pressure at the origin of arterioles within optimal levels. The vasodilator reserve of arterioles distal to constricted pre-arteriolar vessels is reduced because they are already dilated to preserve flow.

In conduit coronary arteries, the pressure remains similar to aortic pressure, but decreases progressively across pre-arteriolar vessels in proportion to their degree of constriction **(A, lower panel).** During metabolic or pharmacological arteriolar vasodilatation, the pressure drop increases only slightly distal to some pre-arteriolar vessels (a_1, a_2, b_2) because vasodilatation related to flow-mediated release of endothelium-derived relaxing factor compensates nearly completely for the increased flow. The pressure drop increases markedly across more constricted pre-arteriolar vessels (b_1, c_1, c_2) **(B, lower panel)**. Steal can develop when an increase in flow through c_2 causes a pressure decrease at the branching point distal to the constricted segment c, so that the driving pressure becomes insufficient to perfuse adequately the most constricted branch c_1 and blood flow (as indicated by arrows) can become lower than during rest conditions. The possibility of flow steal is greatly enhanced if branch c_2 perfuses subepicardial layers of the ventricular wall with a greater flow reserve and if branch c_1 perfuses perfuses the subendocardial layers with a smaller flow reserve. At the end of the pre-arteriolar vessels with the most marked increase in tone (b_1), intravascular pressure may become insufficient to maintain lumen patency, resulting in vessel collapse (From Maseri et al, 1991).

autoregulatory range. With such a change, the myogenic response in the pre-arteriolar vessels may be reset as they are more sensitive to intravascular distending pressure because of their larger size (Chilian et al, 1986a). This resetting would allow arteriolar pressure to remain within its usual optimal range for matching supply to demand. This mechanism may be insufficient to maintain flow at low perfusion pressures, such as in the presence of a severe epicardial stenosis, where distending pressure and myogenic tone are markedly reduced. This situation leads to a resetting of the composition of the interstitial fluid such that the arterioles move into a permanent state of "vasodilatation", exhausting the coronary vasodilator reserve (Chapter 2, Section 2.3)

ii) *Regulation of myocardial blood flow when myocardial oxygen consumption changes*

Vasodilatory metabolites such as ADP, adenosine, H^+ and lactate vary in concentration in the intracellular and hence extravascular compartments in relation to myocardial metabolic activity (Berne & Rubio, 1979). Increased production of these metabolites occurs with increased myocardial oxygen consumption, and thereby arteriolar vasodilatation is produced and an increase in coronary blood flow occurs with maintained perfusion pressure. Pre-arteriolar vessels vasodilate in response to increased flow velocity in order to maintain optimal pressure across the arteriolar bed. The mechanisms whereby these vessels dilate is uncertain although flow-mediated release of EDRF has been postulated. An inability to vasodilate in response to flow would diminish this metabolically-mediated vasodilatation, particularly in the subendocardium which is more susceptible to a reduction in coronary flow. Thus, the ability of the arteriolar vessels to match flow through resetting of the extravascular fluid composition requires a relatively constant flow at origin.

From this theoretical perspective, it is apparent that alterations in the autonomic modulation of coronary flow and in endothelial function occurring as part of the atherosclerotic process may lead to abnormalities in pre-arteriolar vasodilator responsiveness, and thus, resistive vessel dysfunction. With pre-arteriolar constriction or an inability to vasodilate adequately, and thus compensatory arteriolar vasodilatation, there is a reduction in the coronary flow reserve. In addition, there is also a reduction of pressure at the origin of the arteriolar vessels to maintain flow and a sustained increase in interstitial adenosine concentration.

1.4.2. Autonomic Modulation of Coronary Blood Flow

The autonomic nervous system acts to modulate coronary blood flow through a direct effect on coronary arteries which have a neural supply and through humoral means by the release of adrenaline and noradrenaline. Epicardial coronary arteries account for less than 5% of total coronary resistance under normal conditions (Forman & Kirk, 1980). In the presence of coronary artery disease, the effect of the sympathetic nervous system on epicardial vessels may assume a greater importance because of coronary artery spasm and dynamic coronary artery stenoses (Maseri et al, 1987), and the wide acceptance of the importance of coronary vasomotion in the genesis of stable angina (Deanfield et al, 1983), unstable angina (Vetrovec et al, 1982) and myocardial infarction (Hackett et al, 1987). There are sympathetic neural efferents to coronary vessels as small as 100 μm (Chilian et al, 1989b), which account for at least 50% of coronary resistance. It remains entirely likely that alterations in autonomic nervous system activity at the level of the coronary resistive vessels could account for altered coronary blood flow in pathological disease states.

1.4.2.1. α-Adrenergic Modulation of the Coronary Circulation

1. Resistive Vessels

Experimentally, it has remained difficult to demonstrate isolated α-adrenergic vasoconstriction in the coronary resistive vessels because of the effect of sympathetic nervous stimulation/noradrenaline infusion on heart rate and contractility, which lead to an increase in myocardial oxygen demand and metabolic vasodilatation (Section 1.4.1.3). β-blockade as pre-treatment may diminish these cardiac effects but does not remove the effect of the metabolic state on coronary autoregulation. Selective α_1-adrenergic agonists such as phenylephrine and methoxamine may induce vasoconstriction in the coronary circulation (Williams & Most, 1980) and it is generally accepted that stimulation of coronary α-adrenergic receptors causes vasoconstriction, and may limit metabolic vasodilatation by 30% (Mohrman & Feigl, 1978). This is not necessarily a detrimental effect as α-adrenergic vasoconstriction may lessen transmural steal during coronary hypoperfusion distal to a significant epicardial stenosis. When flow becomes pressure-dependent (below the autoregulatory range), α-adrenergic vasoconstriction in the subepicardium lessens steal from the subendocardium presumably by competing more effectively with metabolic vasodilatation in the subepicardium where ischaemia is less severe (Feigl et al, 1987).

Coronary vasoconstriction occurs through stimulation of both post-synaptic α_1- and α_2-adrenergic receptors (Woodman & Vatner, 1987). α_1-adrenergic vasoconstriction occurs throughout the coronary microcirculation but predominately in the small arteries up to 250μm in diameter ("pre-arterioles"), whereas α_2-adrenergic vasoconstriction predominates in arterioles <100μm (in the absence of autoregulatory escape) (Chilian et al, 1989b). However, in the presence of intact autoregulatory mechanisms, dilatation occurs in the small arterioles (Chilian, 1991). The magnitude of arteriolar α_2-adrenergic vasoconstriction is three-fold that of α_1-adrenergic

vasoconstriction. This indicates a preferential number of α_2-adrenergic receptors on small downstream coronary arterioles, although the exact density and function of both receptor subtypes on the endothelium and vascular smooth muscle remains uncertain (Chilian, 1991). Thus, in summary, there is a heterogeneous response to α-adrenergic activation in the coronary microcirculation.

Alpha-adrenergic constriction has been implicated in the causation of myocardial ischaemia distal to a severe epicardial stenosis which may be due to α_1- (Jones et al, 1986) or α_2-mediated vasoconstriction (Heusch & Deussen, 1983). Reflex activation of the sympathetic nervous system eg. following carotid sinus hypotension, elicits peripheral vasoconstriction, increases cardiac contractility and heart rate but with predominant coronary vasodilatation through increased cardiac metabolism with a component of (relative) α-adrenergic constriction (Feigl, 1967). With the converse, α-adrenergic constriction is withdrawn, again with coronary vasodilatation. The central modulation of this reflex arc is not well understood.

The role of reflex α-adrenergic vasoconstriction in the human coronary vascular bed is of importance in elucidating the mechanism of enhanced vasoconstriction which occurs in resistive vessels (Mudge et al, 1976) and in epicardial vessels (Raizner et al, 1980) in patients with coronary artery disease. Studies of animals undergoing exercise have confirmed that α-adrenergically mediated vasoconstriction which attenuates exercise-induced metabolic vasodilatation arises predominately from circulating catecholamines (Chilian et al, 1986b). This vasodilatation is modulated by α_1-adrenergic vasoconstriction and by pre-synaptic α_2-adrenergically mediated re-uptake of noradrenaline (Hendrickx et al, 1984). Thus, α-adrenergic vasoconstriction acts as a brake to excessive vasodilatation such that myocardial function may be limited. The afferent stimulus to this increase in α-adrenergic tone on exercise may come from arterial baroreceptors, chemoreceptor reflexes and from central

neurological sites, with an additional reflex arc from skeletal muscle afferents (Aung-Din et al, 1981).

The role of the sympathetic nervous system in the control of basal coronary blood flow is less well understood. However, in man, α-adrenergic receptor blockade causes an increase in basal coronary blood flow perhaps implying tonic sympathetic control of basal coronary flow (Orlick et al, 1978). In experimental studies however, it remains controversial whether or not regional myocardial sympathectomy leads to an increase (Holtz et al, 1977) or to no change in basal flow (Chilian et al, 1981) and its transmural distribution. In conscious dogs, α-receptor blockade increases coronary flow by only 30% (Macho et al, 1982), less than seen with other interventions, suggesting that α-adrenergic sympathetic tone contributes in part to maintenance of coronary flow at rest.

Recent work has demonstrated an inter-relationship between α-adrenergic vasoconstriction and endothelial-dependent vasodilatation in the control of the coronary microcirculation and which strengthens the hypothesis of pre-arteriolar resistive vessel dysfunction as a mechanism for ischaemia in coronary artery disease. In this study, Jones et al demonstrated in the beating dog heart model that coronary microvascular vasoconstriction by both α_1- and α_2-adrenergic activation was accentuated by inhibition of nitric oxide synthesis (Jones et al, 1993). This implied that nitric oxide release modulated resting coronary microvascular tone, opposing the constrictor action of catecholamines. It has been previously demonstrated that α_2-adrenergic activation may induce endothelial-dependent vasodilatation in isolated canine arteries (Cocks & Angus, 1983) and that both control mechanisms may interact in the systemic circulation (Ohyanagi et al, 1992). In the normal state, the mechanism whereby the endothelium may modify α-adrenergic vasoconstriction is through nitric oxide release through the shear stress of increased coronary flow, thus limiting the reduction in myocardial perfusion induced by this increased sympatho-adrenal drive.

In pathological states such as hypertension (Panza et al, 1990), hypercholesterolaemia (Drexler et al, 1991), diabetes (Saenz de Tejada et al, 1989) and ultimately atherosclerosis (Zeiher et al, 1991), it is possible to see how endothelial dysfunction may lead to the unopposed action of α-adrenergic vasoconstriction on the coronary microcirculation and subsequent pre-arteriolar resistive vessel dysfunction (Section 1.4.3).

2. Epicardial Vessels

The significance of α-adrenergic tone in the vasomotor control of large epicardial vessels is unclear. Even during α-adrenergic activation through stellate ganglion stimulation and noradrenaline infusion in dogs, constriction of large coronary arteries is only sufficient to increase their contribution to coronary resistance to 10% (Kelley & Feigl, 1978). Alpha-adrenergic receptor selectivity is controversial and appears to be species-specific. In dogs, α_1-adrenergic constriction predominates in epicardial vessels (Heusch et al, 1984), whereas both α_1- and α_2-adrenergic constriction of large arteries occurs in calves (Young et al, 1988). Of greater interest, α_2-adrenergic receptors are present on the endothelial cell surface and their stimulation may mediate the release of endothelium-derived relaxant factor (Cocks & Angus, 1983). In the normal human coronary circulation, α_1-adrenergic stimulation reduces flow (and epicardial diameter) but α_2-adrenergic blockade does not alter flow, implying no basal α_2-adrenergic vasoconstrictor tone (Indolfi et al, 1992). In contrast, in patients with coronary artery disease, α_2-adrenergic blockade with yohimbine reduced coronary flow, possibly through noradrenaline stimulation of post-synaptic α_1-adrenoceptors (Indolfi et al, 1992).

Elevated sympathetic tone has been implicated in the causation of epicardial coronary vasospasm (Yasue et al, 1974; Mudge et al 1976; Raizner et al, 1980). Studies have shown that subcutaneous methacholine or adrenaline may induce

coronary vasospasm demonstrated by ST segment elevation (Yasue et al, 1974) and at angiography (Yasue et al, 1976) and may be prevented by phenoxybenzamine in patients with variant (Prinzmetal) angina. Increased sympathetic activity has also been implicated in the genesis of coronary spasm in patients with variant angina undergoing angiography during cold pressor (Raizner et al, 1980), although autonomic stimulation did not induce angina in patients who did not have variant angina. However, trials with prazosin (the selective α_1-adrenergic antagonist) (Winniford et al, 1983) or phentolamine (Chierchia et al, 1984) failed to show a reduction in ischaemic episodes in patients with variant angina. Furthermore, cold pressor, phenylephrine and noradrenaline were ineffective in eliciting vasospasm despite a positive ergonovine stress test (Chierchia et al, 1984). It may be that autonomic dysfunction is a highly localised phenomenon occurring in large vessels perhaps where there is localised atheromatous plaque with associated endothelial rupture and denudation and standard methods of detection of ischaemia are insufficient in demonstrating this interaction between the α-adrenergic component of sympathetic activity and coronary artery disease.

1.4.2.2. β-Adrenergic Modulation of the Coronary Circulation

1. Resistive Vessels

β-adrenergic receptor stimulation may dilate resistive vessels by two mechanisms: i) direct stimulation of vascular receptors, and ii) by increasing tissue metabolic demand. β-adrenergic receptor activation leads to primary resistive vessel dilatation measured as an increase in coronary sinus oxygen saturation with isoprenaline (Klocke et al, 1965). With β_1-receptor blockade, although heart rate and contractility may be reduced, the coronary vasodilator response to isoprenaline remains, concluding that the major mechanism is through β_2-receptor activation, confirmed by direct β_2-receptor stimulation (Vatner et al, 1982). Additional work has suggested that

direct β_1-receptor stimulation may also dilate coronary resistive vessels (Vatner et al, 1984). However, there is little evidence to show that electrical stimulation of sympathetic nerves leads to β_2-mediated vasodilatation (Hamilton & Feigl, 1976).

2. Epicardial Vessels

The functional importance of β-adrenergic receptors in the control of epicardial coronary vasomotion is unknown. Larger epicardial coronary arteries are less sensitive to β-adrenergic stimulation: canine coronary arteries less than 500 μm dilate to adrenaline/noradrenaline whereas vessels greater than 1.5 mm constrict suggesting a segmental divergence of α- and β-receptors in the coronary vascular bed (Züberbuhler & Bohr, 1965). However, the response to neural activation of β-receptors in vivo is not known. The predominant subtype in epicardial vessels is the β_1-receptor, with β_2-receptors predominating in the peripheral circulation. As described above, it is difficult to distinguish between direct β-adrenergic activation/inhibition and the indirect effects of changes in coronary blood flow and metabolism on the regulation of epicardial coronary artery diameter (Macho et al, 1981). When coronary flow is held constant, both β_1- and β_2- selective agonists (prenalterol and pirbuterol, respectively) induce epicardial vasodilatation (Vatner et al, 1986).

The effect of β-blockade on coronary artery diameter and coronary flow is controversial. It has been reported that non-selective β-blockade with propranolol worsens variant angina (Yasue et al, 1974), although it is no longer believed that this is due to unopposed α-adrenergic vasoconstriction (Vatner & Hintze, 1983). Coronary resistance is increased after propranolol in patients with coronary artery disease (Kern et al, 1983) which suggests that irrespective of any effect on large coronary arteries, β-receptors have a role to play in the coronary resistive vessels, even if to counter the underlying effect of the α-adrenergic system on the coronary

resistive vessels under normal circumstances and in patients with coronary artery disease.

1.4.2.3. Cholinergic Modulation of the Coronary Circulation

1. Resistive Vessels

The role of the parasympathetic nervous system in the control of the coronary circulation remains the least well defined of all the constituent parts of the autonomic nervous system. As with the β-adrenergic component of the sympathetic nervous system, both coronary perfusion pressure and myocardial metabolic demand may be altered by parasympathetic stimulation. In addition, the neural and humoral components are different and wide differences exist between species. In anaesthetised dogs, controlling for heart rate, left ventricular pressure, and arterial pressure, parasympathetic activation by acetylcholine administration or vagal stimulation leads to coronary vasodilatation (Feigl, 1969). In contrast, in the calf model, there is initial vasoconstriction followed by dilatation with acetylcholine (Young et al, 1987). However, in other species such as primates, acetylcholine causes vasoconstriction (Sakai, 1981). Much of this difference may be explained by the balance between the endothelium-dependent vasodilator and the vascular smooth muscle constrictor components of acetylcholine.

2. Epicardial Vessels

Acetylcholine has a dual action in the coronary circulation. It is an endothelium-dependent vasodilator at lower molar doses, but at higher doses, its direct smooth muscle constrictor action becomes more prevalent (Furchgott & Zawadzki, 1980). In the presence of endothelial dysfunction, the vasoconstrictor action persists. For this reason, the major interest in clinical studies remains the role of the cholinergic system

in the vasoconstrictor response of atherosclerotic coronary arteries to acetylcholine (Ludmer et al, 1986).

There is little evidence that there is significant cholinergic neural activation of the epicardial vessels, despite a rich parasympathetic innervation of the adventitia of these arteries. It has been suggested that these nerves may modulate noradrenaline release from sympathetic nerve endings (Vanhoutte et al, 1981), although there is no evidence for this in humans (Kalsner, 1985).

1.4.3. Endothelial Modulation of Coronary Blood Flow

Endothelial cells produce and release factors which play a central role in the local control of the circulation in any vascular bed. Both vasodilator and vasoconstrictor substances are produced and under normal conditions, vasodilatation predominates (Figure 1.4). In the presence of atherosclerosis and coronary artery disease, this balance may be upset such that vasoconstrictive factors dominate and the anti-platelet and anti-thrombotic properties of normally functioning endothelium are lost.

Since Furchgott and Zawadzki published their initial findings describing endothelium-dependent vasodilatation to acetylcholine (Furchgott and Zawadzki, 1980), there has been a large body of research published defining the role of the endothelium and its modulation of vascular smooth muscle tone and the control of coronary blood flow. The major humoral factor which modulates this vasodilatation is called endothelium derived relaxing factor (EDRF) and is thought to be nitric oxide (Palmer et al, 1987), although it has been suggested that in vivo it may be a closely related molecule such as S-nitrosocysteine, a nitrosothiol (Myers et al, 1990). It is produced by endothelial cells from L-arginine by the enzyme, nitric oxide synthetase (Palmer et al, 1988), but has a very short half-life limiting its effect to the immediate vascular smooth muscle. EDRF may be released in response to a variety of different stimuli; flow (shear stress), platelet-derived products (ADP, thrombin, serotonin) and

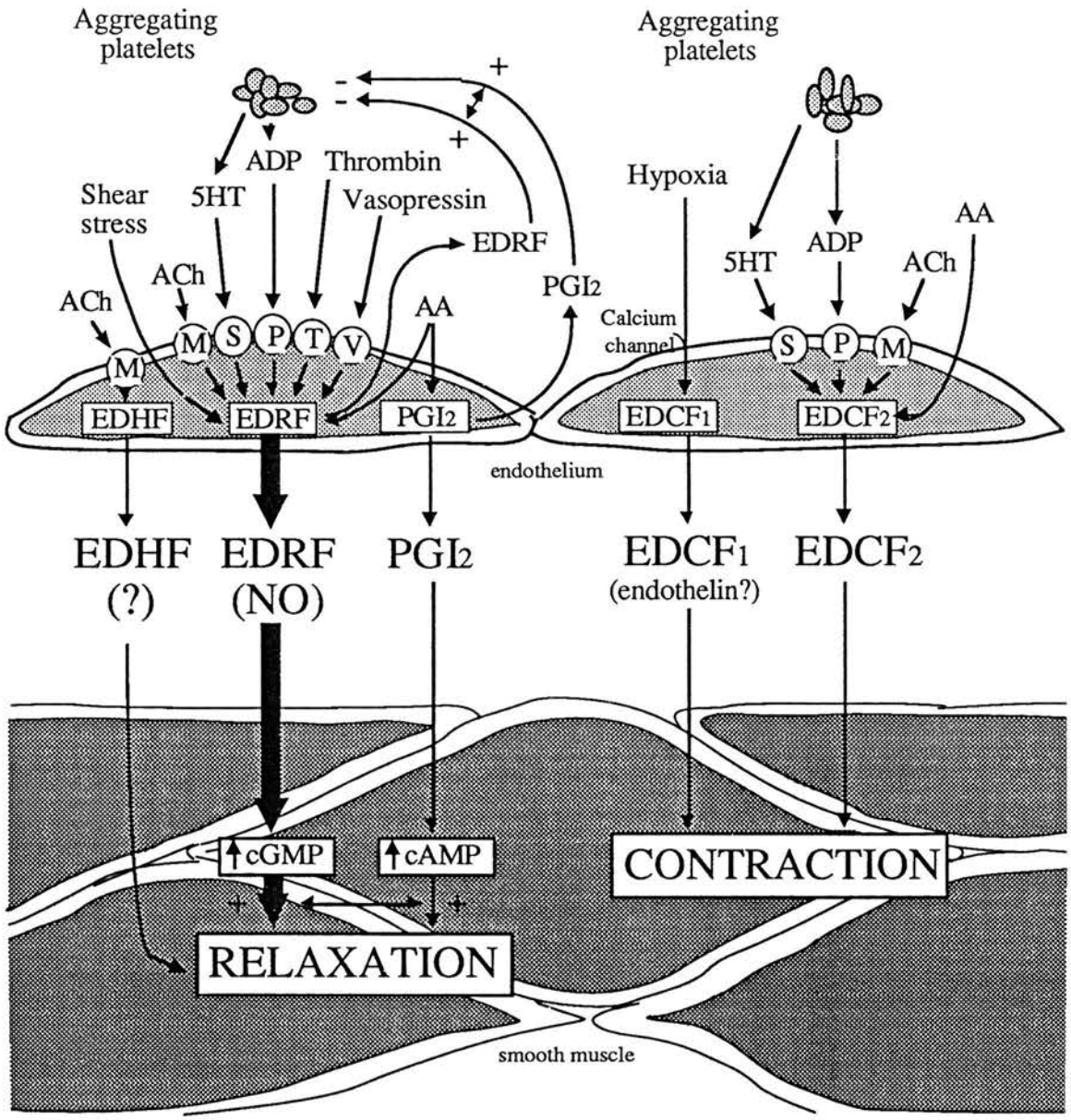


Figure 1.4. Overall view of endothelium-derived factors and modulation of vascular smooth muscle contraction. Endothelium-derived relaxing factor (EDRF) increases cyclic GMP (cGMP) levels through activation of soluble guanylate cyclase. Prostacyclin (PGI_2) is another vasodilator released from the endothelium which stimulates adenylate cyclase, and increases cyclic AMP (cAMP) levels. EDRF and prostacyclin could act synergistically to relax vascular smooth muscle and inhibit platelet aggregation. Endothelial cells also produce a hyperpolarising factor (EDHF) which is probably a metabolite of arachidonic acid, such as an endoxide or lipoxide, and which is also a vasodilator. Two endothelium -derived contracting factors exist; the first endothelial-derived contracting factor ($EDCF_1$) may be endothelin, and $EDCF_2$ may be superoxide anions. ACh = acetylcholine, 5-HT = 5-hydroxytryptamine (serotonin), ADP = adenosine diphosphate, AA = arachidonic acid. M = muscarinic receptor, S = serotonergic receptor, P = purinergic receptor, T = thrombin receptor, V = vasopressinergic receptor (Modified from Vanhoutte & Shimokawa, 1989).

hormones/vasoactive products (bradykinin, histamine, noradrenaline, substance P, vasopressin) all stimulate production and release of EDRF. It acts on vascular smooth muscle by activating guanylate cyclase to increase intracellular levels of cyclic guanosine 3',5'-monophosphate (cGMP). This in turn inhibits agonist-induced activation of the phosphoinositol pathway, which normally increases free calcium in the cytosol (Vanhoutte & Shimokawa, 1989).

The interaction between endothelium and platelets is complex (Figure 1.4). Prostacyclin (PGI_2) is produced in the main by endothelial cells (Moncada & Vane, 1979) and is a potent inhibitor of platelet aggregation with a direct vasodilator effect on vascular smooth muscle. Its mechanism of action involves the increased production of cyclic adenosine 3',5'-monophosphate (cAMP). Low doses of PGI_2 and EDRF enhance the anti-aggregatory and vasodilator action of each other. EDRF is a potent inhibitor of platelet adhesion and aggregation, and the release of ADP from platelets induces further EDRF release. Serotonin and thromboxane A_2 act directly on vascular smooth muscle as vasodilators. In the coronary circulation, this interaction with platelets in the presence of intact or normally functioning endothelium protects against vasoconstriction, thrombus formation and the likelihood of myocardial ischaemia.

Another endothelial vasoactive compound is endothelium-derived hyperpolarising factor (EDHF) (Vanhoutte & Shimokawa, 1989). This compound is less well defined than EDRF and its existence is suggested by the demonstration that endothelium-dependent relaxation to acetylcholine and the effect of acetylcholine on vascular smooth muscle membrane potential is reduced by ouabain, an inhibitor of the sodium-potassium ATPase, and independent of an EDRF action. EDHF may contribute to a more sustained vasodilator action and thus complement the action of EDRF. In certain circumstances, the endothelium may release factors which cause vasoconstriction. Endothelin is a potent vasoconstrictor peptide (Yanagisawa et al, 1988) which may cause prolonged contraction of vascular smooth muscle (Hirata et al, 1988). Whether

the endothelium-derived contracting factor described in hypoxic canine arteries is endothelin is uncertain (Rubanyi & Vanhoutte, 1985). High local concentrations of angiotensin II may also be produced by the endothelium (Dzau, 1986), which could contribute to predominant vasoconstriction. Thus, in pathological states these vasoconstrictor compounds could override the normal vasomotor tone associated with endothelium-dependent vasodilatation (Lüscher, 1989) (Figure 1.5).

1.4.3.1. Endothelium and Coronary Autoregulation

Although EDRF release is a universally demonstrated constituent of all vascular beds, its activity varies in different vascular beds in terms of basal release, flow-induced release and release by specific vasoactive compounds. This variability is compounded by the fact that endothelial-active agents, acetylcholine for example, may have a vasoconstrictor action on vascular smooth muscle.

Although much research into the physiological properties of EDRF has used isolated vascular rings (from large vessels) in organ baths, it is the role of EDRF in the control of the coronary microcirculation and on resistive vessel function that is of most relevance in the study of the coronary microcirculation. By infusing an inhibitor of nitric oxide production, forearm blood flow may be reduced implying tonic release of EDRF as a modulator of basal arteriolar tone (Vallance et al, 1989). In the coronary circulation, infusion of an inhibitor of nitric oxide production caused a small but significant reduction in coronary blood flow in normal coronary arteries (Lefroy et al, 1993), indicating a basal release of nitric oxide in the coronary microcirculation to maintain resting coronary flow.

A major determinant of EDRF release at the level of the coronary resistive vessels is that of coronary flow itself which acts through shear stress on the endothelial cells. The myogenic response which may be a component part of coronary autoregulation (Section 1.4.1) would be uncontrolled ie, inappropriate vasoconstriction, were it not

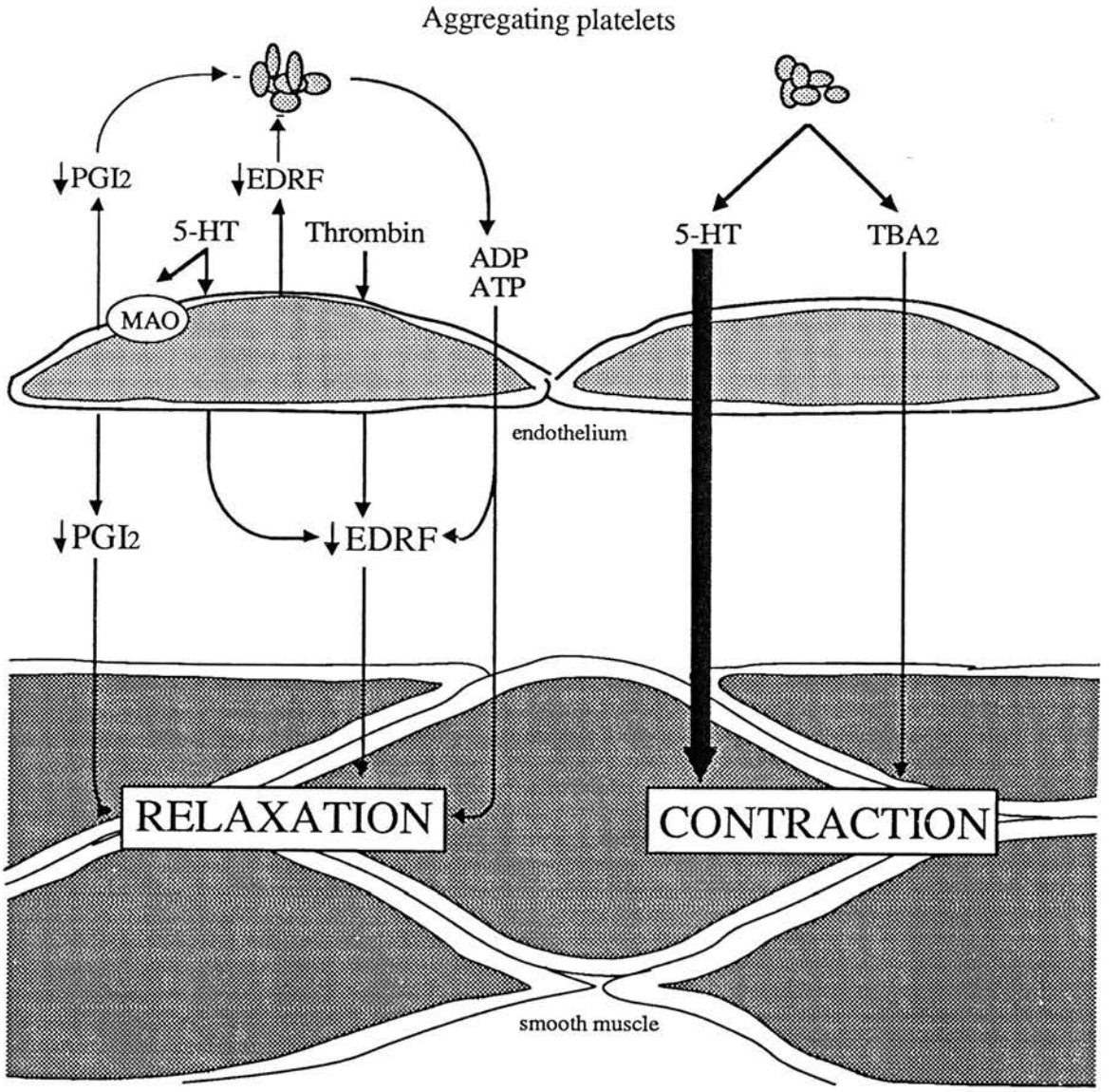


Figure 1.5. Endothelium-dependent responses under pathological conditions such as hypercholesterolaemia and atherosclerosis during platelet aggregation. Normally, EDRF inhibits platelet aggregation and adhesion as well as platelet-induced vasoconstriction. Less EDRF is released leading to a imbalance of vascular smooth muscle contraction, secondary to serotonin and thromboxane A_2 acting on smooth muscle independent of endothelium. In atherosclerosis, both EDRF and prostacyclin production are reduced, with little synergistic action against platelets. Abbreviations as Figure 1.4, except ATP = adenosine triphosphate, TBA_2 = thromboxane A_2, MAO = monoamine oxidase (Modified from Vanhoutte & Shimokawa, 1989).

for the release of EDRF in response to the initial increase in flow occurring in response to the increase in perfusion pressure. Thus, the degree of successful autoregulation by the coronary resistive vessels is dependent on an intact normally functioning endothelium and adequate production or release of nitric oxide (Griffith & Edwards, 1990). It is of interest that the greatest amount of EDRF activity resides in the pre-arteriolar vessels (>100 μm) which are under the most shear stress (Griffith et al, 1987). Given the importance of these vessels in distributing perfusion to meet tissue needs in the context of varying perfusion pressure, the integrity of EDRF activity would seem to be important in avoiding the disturbance of myocardial perfusion, occurring as an early manifestation of coronary artery disease (Section 1.4.3.2.2).

The heterogeneous response of the coronary resistive vessels to nitroglycerin is further evidence for a functional distinction between pre-arteriolar and arteriolar vessels. Nitroglycerin dilates resistive vessels >100 μm, but not the arterioles <100μm because of the latter's inability to convert nitroglycerin to its vasoactive metabolite. This occurs because the arterioles lack sulphydryl-containing compounds such as cysteine to convert the nitroglycerin to a nitrosothiol, with subsequent conversion to nitric oxide (Kurz et al, 1991). In this study, it was suggested that arteriolar vascular smooth muscle cells have a markedly reduced sulphydryl "pool" compared to larger vessels accounting for this heterogeneous vasodilator response.

1.4.3.2. Endothelial Function and Disease States

Because of the central role played by the endothelium in the control of tissue perfusion, its function may be affected by the risk factors for cardiovascular disease. This endothelial dysfunction may be the earliest manifestation of the disease process and account for many of the abnormalities documented in regional tissue perfusion.

1. Cardiovascular Risk Factors and Atherosclerosis

Risk factors such as hypercholesterolaemia and hypertension lead to an impairment of endothelial function as has been shown in many different experimental models. In a rabbit aorta model, low-density lipoprotein (LDL) inhibits endothelial-dependent relaxation to acetylcholine (Andrews et al, 1987) as well as prostacyclin production (Nordoy et al, 1978). Although LDL can impair EDRF activity directly, the more prolonged effect of oxidised LDL may be on the cell membrane of the endothelial cell diminishing receptor-mediated EDRF release (Kugiyama et al, 1990). As this may have the effect of increasing local concentrations of LDL on the intimal side of the media (Curmi et al, 1990), it is interesting to speculate that this may lead to the local development of atheroma.

With the development of atheroma, endothelial-dependent relaxation to a variety of different vasoactive agents is impaired (Verbeuren et al, 1986; Freiman et al, 1986; Shimokawa et al, 1988). Acetylcholine infusion may even cause paradoxical vasoconstriction in patients with coronary artery disease and in transplant patients (Ludmer et al, 1986). In atherosclerosis, these changes may be accounted for mainly by reduced EDRF release, but also by intimal and media changes, and often release of an EDCF (Bossaller et al, 1987; Shimokawa et al, 1987). There is now much evidence to show that the vasodilator responsiveness of the coronary resistive vessels may be impaired not only by the atherosclerotic process but in the presence of hypercholesterolaemia alone (Section 1.5).

In experimental models of hypertension, endothelium-dependent relaxation to acetylcholine, ADP, and serotonin (Lamping & Dole, 1987) is impaired. Moreover, there may be a relative excess of endothelin over EDRF in the resistive vessels of hypertensive circulations compared to normal (Lüscher et al, 1990). In clinical studies, impaired endothelial-dependent vasodilatation has been described in the forearms of patients with essential hypertension (Panza et al, 1990).

2. Coronary Artery Disease

In experimental models, hypoxia and ischaemia inhibit EDRF production and release (De Mey & Vanhoutte, 1983) and there is much clinical evidence to show that endothelial dysfunction may contribute to myocardial ischaemia. Some patients with localised epicardial coronary artery stenoses may sustain coronary spasm sufficient to cause myocardial ischaemia (Maseri et al, 1975) and local endothelial dysfunction has been implicated as the mechanism behind this phenomenon (Kaski et al, 1986). Coronary spasm will occur at sites of endothelial denudation in response to agents which normally cause endothelial-dependent vasodilatation but have direct vasoconstrictor effects on vascular smooth muscle such as acetylcholine (Yasue et al, 1986), histamine (Shimokawa et al, 1983) and serotonin (Brum et al, 1984; Shimokawa et al, 1987). In the clinical setting, platelet activation will release endothelial-active agents such as serotonin, ADP and thrombin which may contribute to this unopposed vasoconstriction in patients with variant angina.

Much more commonly, coronary artery disease is a diffuse process with more than one discrete lesion affecting some or all of the main epicardial coronary arteries with atheromatous lesions which are of variable morphology with a variable dynamic component to each lesion. This complicated process will lead to a variable degree of endothelial dysfunction in the epicardial coronary artery of interest and endothelial denudation may exacerbate this situation. It has been demonstrated convincingly that diseased epicardial coronary arteries will vasoconstrict to infusion of acetylcholine (Ludmer et al, 1986) and serotonin (McFadden et al, 1991) and that with the latter agent, the degree of vasoconstriction is greatest in the smallest epicardial arteries. In porcine coronary arteries, endothelium denuded by gentle balloon injury regenerates but with a response to endothelial-dependent vasodilators which remains impaired for a prolonged period of time, and in this porcine model, with release of an excess of an endothelium-derived constricting factor (Shimokawa et al, 1987). In addition to

vasoconstriction of epicardial arteries with endothelial dysfunction, the inhibitory effect of EDRF on platelet adhesion and aggregation is lost (Hogan et al, 1988), exacerbating the effect of serotonin released from platelets and leading to a reduction in coronary blood flow by an effect on the microcirculation (Golino et al, 1991).

In summary, the endothelium may undergo a functional change in response to the physiological environment. This functional change may manifest as altered vasdilator responsiveness which is only one component of the multifarious role of the endothelium. Impaired endothelial function will also lead to an increase in leucocyte adhesion and platelet aggregation and an alteration in the control of vascular smooth muscle tone. There is a strong relationship between the coronary endothelium and the atherosclerotic process. It is likely that the alteration in endothelial function which occurs alters coronary resistive vessel function sufficient to cause change in their role in coronary autoregulation and vasodilator responsiveness to pharmacological stimuli.

1.5 Resistive Vessel Dysfunction in Coronary Artery Disease

Myocardial ischaemia occurs when perfusion pressure is so low at the origin of maximally dilated arteriolar vessels that increased myocardial oxygen extraction cannot compensate for the reduced coronary flow. This usually occurs in the presence of a severe epicardial coronary artery stenosis. However, there are several observations in experimental models and clinical studies to suggest that myocardial ischaemia may also be caused by resistive vessel (pre-arteriolar) constriction. In this setting, myocardial ischaemia on exercise may occur by two mechanisms i) an increase in myocardial oxygen consumption above the coronary flow reserve and ii) arteriolar vasodilatation with a reduction in intraluminal distending pressure, insufficient to oppose extravascular pressure.

The vasoactive peptides, neuropeptide Y and endothelin have been shown to cause myocardial ischaemia in humans (Clarke et al, 1987) and conscious dogs (Larkin et al, 1989) respectively, with no demonstrable effect on epicardial coronary arteries. Myocardial ischaemia may also occur through resistive vessel constriction following high doses of intracoronary acetylcholine in angiographically normal coronary arteries (Newman et al, 1990) and with serotonin in patients with coronary artery disease (McFadden et al, 1991). This latter effect may also be exacerbated by the vasoconstrictor action of serotonin on intramural penetrating arteries (mean diameter 200 μm) in the presence of a significant proximal stenosis, thus aggravating subendocardial ischaemia (Bache et al, 1992). After emergency angioplasty, ischaemia may occur due to microvascular constriction (Wilson et al, 1989) and this vasoconstriction may be seen in visible distal vessels after an elective procedure (Fischell et al, 1990). Even in patients with chronic stable angina, it has been shown that inappropriate resistive vessel constriction may cause or contribute to causing myocardial ischaemia (Pupita et al, 1990).

For inappropriate resistive vessel constriction to occur in the presence of myocardial ischaemia, it is implicit that the potent compensatory vasodilator stimulus of local metabolites is overcome, lending weight to the hypothesis that there is a distinct functional heterogeneity of resistive vessels (Section 1.4.1.4). Although it remains a possibility that inappropriate constriction of arteriolar vessels could account for the resistive vessel constriction, this is most unlikely as it would require an unrealistic constrictor stimulus in the presence of potent vasodilator metabolites such as H^+ and a low tissue pO_2.

There is increasing evidence to demonstrate that the atherosclerotic process affects the coronary resistive vessels. In a recent study of the microcirculation using normal and atherosclerotic monkeys, Chilian et al showed that serotonin (an endothelium-dependent vasodilator) decreased coronary resistance in normal animals but increased

large artery and microvascular resistance in atherosclerotic animals (Chilian et al, 1990a). Ergonovine, although elevating microvascular resistance in both animal groups, increased large artery resistance in the atherosclerotic group. Atherosclerosis however did not potentiate the response to phenylephrine (an α1-adrenergic agonist). Given that the response to adenosine was no different in the two groups, this implied that atherosclerosis did not impair vascular smooth muscle responsiveness. In conclusion, this study demonstrated that the atherosclerotic process was associated with endothelial dysfunction. As the endothelium modulates microvascular responses to vasoactive substances (Förstermann et al, 1987), and hypercholesterolaemia has been shown to impair endothelial-dependent relaxation to ATP and acetylcholine in larger arteries (Verbeuren et al, 1986), it was suggested that a similar mechanism could occur in the microcirculation.

To investigate further the mechanism of effect of atherosclerosis on microvascular function, isolated cannulated coronary arterioles (30-70 μm) from atherosclerotic and normal pigs were studied using the endothelial-dependent agents, ADP, serotonin and histamine (Kuo et al, 1992). These agents induced vasodilatation of only 20-30% of controls, even then only at the highest doses. The response to flow was abolished in denuded normal arterioles and in those from atherosclerotic animals. However, administration of L-arginine (the substrate for synthesis of EDRF) restored to normal the vasodilator response to pharmacological stimulation and to flow. In hypercholesterolaemic animals, it appears that the availability of L-arginine is the rate-limiting factor in EDRF production (Rubanyi, 1991), and it is thought that the oxidised low density lipoprotein (LDL) cholesterol accumulating in the vascular wall interferes with receptor-mediated release of L-arginine from intracellular stores or the synthesis of the amino acid (Tanner et al, 1991). Thus, it was concluded that the abnormality in arteriolar responsiveness developing with large vessel disease involved

an impairment of the synthesis and/or release of the endothelium-derived relaxing factor (Kuo et al, 1992).

The atherosclerotic process appears to have a similar effect on coronary vasomotor tone in man. It has been demonstrated previously that normal epicardial vessels dilate in response to increasing flow induced by the effect of intracoronary papaverine on the resistive vessels (Drexler et al, 1989). In the same study however, this flow-dependent coronary vasodilatation was reduced in atherosclerotic arteries. Zeiher et al studied the coronary vasomotor responses to three different endothelium-mediated stimuli; acetylcholine, increasing blood flow, and sympathetic activation by cold pressor testing in different groups of patients (Zeiher et al, 1991). All three stimuli elicited epicardial artery dilatation in patients with angiographically normal coronary arteries and no cardiovascular risk factors. Subjects with normal arteries but hypercholesterolaemia sustained vasoconstriction to acetylcholine. Patients with a normal artery but with disease elsewhere showed both vasoconstriction to both acetylcholine and cold pressor, with preservation of flow-dependent vasodilatation. Patients with angiographic wall irregularities showed abolition of vasodilatation to all three stimuli, thus indicating a hierarchy of impairment on endothelial-dependent vasodilatation in epicardial arteries with progression of the atherosclerotic process. With respect to the microcirculation, the dilator response to acetylcholine was markedly reduced in patients with smooth epicardial arteries and hypercholesterolaemia, compared to the other groups. Patients with smooth arteries but disease elsewhere also showed a reduced response to acetylcholine, and this was associated with those patients with raised LDL cholesterol. However, the dilator response to papaverine (flow-dependent vasodilatation) was lower only in those with macroscopic disease of the study artery compared to the control group. Cold pressor testing decreased vascular resistance in normals and those with hypercholesterolaemia, but it was increased in those with disease (Zeiher et al, 1991).

In patients with hypercholesterolaemia and minimal irregularities of the epicardial vessel wall, it is also possible to reverse this impairment in endothelial-dependent resistive vessel dilatation with L-arginine. Using Doppler catheterisation, Drexler et al have shown that infusion of L-arginine before intracoronary acetylcholine can restore the vasodilator response of the myocardial region under study to that in normocholesterolaemic patients (Drexler et al, 1991). In both patient groups, the response to papaverine, an endothelium-independent vasodilator, was not impaired and basal coronary flow was unaffected by L-arginine infusion, implying that it was during vasodilatation that the deficit in EDRF production was most prominent (Drexler et al, 1991).

Thus, there is progressive impairment in endothelium-dependent vasodilatation in patients at different stages of early atherosclerosis. This endothelial abnormality may precede atherosclerosis ie. in the presence of hypercholesterolaemia, or be an early marker for the disease process. That this abnormality may alter the vasodilator responsiveness of the coronary resistive vessels is of central importance to the hypothesis of discordance between pre-arteriolar and arteriolar resistive vessels. Such an impairment could be modulated by an increased sensitivity to neural or humoral vasoconstrictor stimuli (Maseri et al, 1990) and account for insufficient vasodilatation or inappropriate vasoconstriction of the pre-arteriolar vessels.

1.6 Existing Models of Resistive Vessel Dysfunction

There is evidence to suggest that there is altered coronary resistive vessel function in different pathological states which affect the cardiovascular system. In these conditions however, there are several physiological variables which complicate the

study of coronary blood flow and make the demonstration of coronary resistive vessel dysfunction less clear.

1.6.1. Hypertension

Systemic hypertension, by definition, is associated with an increase in the peripheral arterial resistance. However, even before the development of ventricular hypertrophy, the alteration in systemic haemodynamics may affect coronary perfusion pressure and myocardial oxygen demand, both determinants of basal coronary blood flow. Theoretically, in hypertension however, even with a reduction in maximal vasodilatation through hypertrophy, the increased perfusion pressure may normalise the coronary reserve as maximal coronary flow will be comparable for the same (autoregulated) basal flow.

In the presence of left ventricular hypertrophy irrespective of the primary cause, the coronary flow reserve may be reduced. In one study, there was a 34% reduction in the coronary flow reserve with dipyridamole (Strauer, 1979). Maximal vasodilatation may be reduced by up to 50% in such patients and this marked reduction in the coronary reserve can lead to chest pain (Opherk et al, 1984). This reduction in regional myocardial blood flow in patients with left ventricular hypertrophy has been demonstrated using positron emission tomography (Goldstein & Haynie, 1990).

In hypertensive ventricles, the normal transmural distribution of coronary flow is accentuated. In dogs with left ventricular hypertrophy at fast atrial pacing (200 beats·min^{-1}), although flow increased similar to controls, there was a reduction in the endocardial/epicardial ratio of flow (Mueller et al, 1980). At 250 beats·min^{-1}, subendocardial coronary flow reserve was exhausted, but with some residual reserve in the subepicardium (Bache et al, 1981). Thus, resistive vessel function may vary

across the myocardium in any one region of interest. However, current methods of measuring coronary blood flow in man cannot discriminate transmural regional flow.

There are several mechanisms whereby the coronary reserve may be reduced in left ventricular hypertrophy. Increased myocardial demand may increase basal flow with no effect on maximal vasodilatation. The maximal cross-sectional area of the resistive vessels does not generally increase in parallel with left ventricular mass such that flow per gram at maximal vasodilatation may actually diminish (Tomanek et al, 1986). This imbalance is probably most severe when due to a pressure load (Marcus et al, 1983a). Furthermore, with myocardial and vascular hypertrophy there may also be a physiological derangement of coronary blood flow regulation contributing to the reduction in flow reserve (Gradman, 1992). Elevated end-diastolic pressure in left ventricular hypertrophy may also reduce maximal dilatation through extravascular compression, particularly in the subendocardium (Ellis & Klocke, 1979). There are also several additional factors which may determine the degree of impairment of the coronary reserve such as the duration of hypertrophy and the stage of its development (Marcus et al, 1981).

In the absence of hypertrophy, resistive vessel dysfunction may occur perhaps secondary to the high perfusion pressure. Structural changes may occur with hypertrophy of the vascular smooth muscle with subsequent thickening of the medial layer, and thus a disproportionate reduction in luminal diameter in response to any vasoconstrictor stimulus (Vogt et al, 1990). In one study, intravenous ergonovine caused a increase in coronary resistance in patients with hypertension compared to controls, which may suggest increased sensitivity of the pre-arteriolar vessels to circulating vasoconstrictors (Brush et al, 1988). In the human forearm model, the vasodilator effect of acetylcholine infusion is reduced in hypertensive patients, although the response to nitroprusside was the same, implying impaired release or production of EDRF by the forearm resistive vessels (Linder et al, 1990; Panza et al,

1990). In the human coronary circulation, intracoronary acetylcholine causes marked vasoconstriction in epicardial arteries of hypertensive patients with normal vasodilatation to nitroglycerin, although this study did not determine if the abnormality was at the level of the resistive vessels (Brush et al, 1992).

Hypertension and myocardial hypertrophy serve as model for resistive vessel dysfunction although it is likely that this is only one factor contributing to the impairment of coronary flow reserve in the absence of angiographic disease.

1.6.2. Cardiac Transplantation

After successful cardiac transplantation, the incidence of accelerated coronary artery disease increases, with up to 50% of patients demonstrating significant epicardial vessel stenoses by 3 years (Uretsky et al, 1987). The small and medium sized epicardial arteries are also affected by this diffuse process making angiographic assessment more difficult (Gao et al, 1988). The coronary flow reserve may be impaired in transplant patients before the development of angiographic disease however, and with positron emission tomography, it has been shown that this impairment is due to a higher basal flow occurring because of hypertension and thus an increased myocardial oxygen demand (Rechavia et al, 1992). However, maximal vasodilatation to dipyridamole also tended to be lower than in controls. In patients with angiographically normal arteries, the endothelium-dependent epicardial vessel dilatation to substance P is preserved (Kushwaha et al, 1991), although epicardial vessel constriction has been demonstrated to acetylcholine (Fish et al, 1988) Measuring the resistive vessel response to endothelium-dependent vasodilatation in transplant patients with early angiographic disease, Treasure et al showed that there was a sequential reduction each year in the coronary vasodilator response to acetylcholine when compared to the non-endothelium-dependent vasodilator, adenosine (Treasure et al, 1992). It was believed that this reflected both a primary

deterioration in endothelial cell function and underlying vasomotor insensitivity as graft arteriosclerosis progressed. Using the endothelium-independent vasodilator, papaverine, it is possible to show an impaired coronary vasodilator response in transplant patients with minor occlusive disease compared to patients with angiographically normal arteries or controls (Mullins et al, 1992).

Thus, in cardiac transplant patients with early atherosclerosis there is an impairment of the coronary flow reserve. However, given the development of diffuse disease of the distal epicardial vessels (Billingham, 1987), a significant degree of coronary resistance must move proximally to these vessels and reduce the contribution of normal or abnormal resistive vessel function to the impaired flow reserve.

1.6.3. Syndrome X

Syndrome X refers to a condition first defined by chest pain and a positive exercise test in the presence of angiographically normal coronary arteries and the absence of epicardial arterial spasm (Kemp et al, 1973). This controversial condition may serve as a model of resistive vessel dysfunction, as an impairment of vasodilator responsiveness (leading to a reduced coronary flow reserve) has been consistently observed using pharmacological vasodilatation (Opherk et al, 1981; Bortone et al, 1989; Geltman et al, 1990; Camici et al, 1991a) and atrial pacing (Cannon et al, 1983; Camici et al, 1991a). Although the nature of the resistive vessel abnormality remains poorly defined (Cannon et al, 1988), syndrome X may represent be a wider disturbance of resistive vessel function with altered vasodilator reserve also reported in the forearm circulation of these patients (Sax et al, 1987). There is evidence to suggest that endothelial function is altered in syndrome X patients (Motz et al, 1991), and an association has also been suggested between syndrome X and the presence of hyperinsulinaemia (Dean et al, 1991). Modulation of the coronary resistive vessels by the sympathetic nervous system may be also be abnormal as chronic treatment with

oral doxasosin, the α_1-adrenergic antagonist, restores the coronary flow reserve to normal in the subgroup of patients with a reduced reserve (Camici et al, 1991b).

Whether the resistive vessel abnormality gives rise to myocardial ischaemia in syndrome X is much more controversial. In one study, patients experiencing typical pain during pacing after intravenous ergonovine demonstrated a lower flow response (and higher coronary resistance) than those without pain (Cannon & Epstein, 1988). In this group with pain at pacing after ergonovine, there was an increase in myocardial oxygen extraction suggesting an inadequate tissue delivery of oxygen, although only 10% of these patients demonstrated a net lactate production at pacing stress. Although stress tests may cause chest pain and ECG changes characteristic for myocardial ischaemia (>0.1 mV ST segment depression), the development of ventricular wall motion abnormalities on echocardiography during exercise is controversial with conflicting reports of the presence (Cannon et al, 1985) and absence of such changes (Nihoyannopoulos et al, 1991). Using coronary sinus catheterisation and measurement of myocardial substrate extraction, net lactate production at maximal atrial pacing has not been documented despite ECG evidence of ischaemia (Camici et al, 1991a). Furthermore, the chest pain of syndrome X is poorly controlled by standard anti-ischaemic therapy (Cannon et al, 1992). Thus, an impaired coronary flow reserve and myocardial ischaemia are not synonymous when studying resistive vessel function.

In theory, a reduced coronary flow reserve could occur in the absence of myocardial ischaemia if there was a concomitant reduction in cardiac work and thus reduction in myocardial oxygen demand, or an increased extraction of metabolic substrate such as oxygen. Given that left ventricular function remains normal at rest and often after exercise/stress, there is no evidence for a reduction in myocardial oxygen demand. In addition, at pacing, syndrome X patients achieve the same rate pressure product as normals without increasing myocardial oxygen extraction, but

with reduced energy expenditure measured by indirect calorimetry (Camici et al, 1991a).

The problem with syndrome X as a model of resistive vessel dysfunction is that it is a heterogeneous group of patients dependent on inclusion criteria (Cannon et al, 1992), and consists of more than one pathophysiological entity. Thus, patients may have low or normal flow reserve (Camici et al, 1992), an altered perception of pain (Cannon et al, 1990) the presence or absence of left bundle branch block (Opherk et al, 1989), a normal or hyperdynamic response to exercise (Camici et al, 1991a), or the presence or absence of regional wall motion abnormalities on exercise (Cannon et al, 1985). For these reasons, although in a large subgroup of cases of syndrome X, there may be resistive vessel dysfunction occurring secondary to an alteration in sympathetic drive or as a primary microvascular abnormality, as a pure model of coronary resistive vessel function, syndrome X remains a controversial subject for investigation.

1.7 Summary

The coronary resistive vessels are a major determinant of coronary blood flow and myocardial perfusion. There are several physiological parameters which determine the extent of myocardial perfusion under resting conditions and under stress. It has been hypothesised that resistive vessels may be considered as two groups roughly according to vessel size; a proximal compartment controlled by coronary flow, intravascular distending pressure and myogenic tone and modulated by the autonomic nervous system, and a distal compartment mainly influenced by the perfusion pressure at origin and the composition of the interstitial fluid, and thus the metabolic state of the myocardium. Myocardial perfusion and thus coronary resistive vessels are dependent on the integrity and function of the endothelium. There is increasing experimental and clinical evidence for inappropriate vasoconstriction or an an inability to vasodilate fully

in the proximal "pre-arteriolar" coronary resistive vessels. Models exist of abnormal resistive vessel function in patients with hypertension, after cardiac transplantation, and in syndrome X. It is possible that both the effect of the early atherosclerotic process on endothelial function and alterations in autonomic nervous control could account for this abnormality in patients with coronary artery disease.

The intention of this thesis is to document the presence of abnormal coronary resistive vessel function in patients with different manifestations of coronary artery disease using Doppler catheterisation and positron emission tomography and to determine the prevalence and possible mechanisms of this abnormality.

CHAPTER 2. THE MEASUREMENT OF CORONARY BLOOD FLOW

2.1 The Assessment of Coronary Blood Flow

The regulation of coronary blood flow has been studied extensively in animals using experimental techniques which are highly accurate in terms of both temporal and spatial resolution. As described in Chapter 1, techniques have also been developed in animal models to study microvascular flow on the surface of the beating heart. By comparison, many methods developed to study the human coronary circulation are relatively crude and more sophisticated techniques are not widely available in clinical practice. Thus much of what is known about the regulation of the human coronary circulation has been derived from research done on other mammals. Although there is much similarity between species under normal conditions, the regulation of the human coronary circulation in disease is less well understood which accounts for the variety of techniques employed to measure myocardial perfusion in man.

In the human heart, the ability of the resistance vessels to dilate or to constrict can only be assessed indirectly from measurement of coronary blood flow. Coronary blood flow can be measured invasively by Doppler flow catheter and quantitative angiography (Wilson et al, 1985), or by coronary sinus thermodilution (Ganz et al, 1971). However, these techniques measure blood flow through a single coronary artery branch and through the coronary sinus or the great cardiac vein, respectively, and so do not allow the comparison with reference myocardium perfused by normal vessels in the same patient at rest and during interventions (Marcus et al, 1987). Myocardial blood flow (MBF) can also be measured non-invasively by positron emission tomography (PET) (Bergmann et al, 1985). This technique has two important advantages over the above methods. Firstly, it provides a measure of the

regional distribution of flow for the entire left ventricular wall and allows comparison of perfusion in the myocardial region of interest with a region perfused by normal arteries. Thus, it allows the vasodilator or vasoconstrictor response of the vascular segment under study to be compared with the effect of the stimulus on adjacent normal coronary vascular segments. Secondly, being non-invasive, it allows repeated assessment of the vascular response in tissue.

2.2 Limitation of Arteriographic Techniques

The anatomical significance of epicardial coronary artery disease may be documented by analysis of coronary arteriograms. However, large intra- and inter-observer variability exist with visual inspection of arteriograms. Despite the use of computer-assisted edge-detection methods (Brown et al, 1977) to reduce the error and inaccuracy of visual assessment (Hoffman, 1984; Klocke, 1987), poor correlations still exist with post-mortem evaluation of coronary stenoses (Grondin et al, 1974). Furthermore, there is a poor correlation between anatomical estimate of the severity of a coronary stenosis and any physiological measurement of the functional significance of the stenosis (White et al, 1984; Harrison et al, 1984). This is particularly true for lesions in the range of diameter stenoses 50-90% (Klocke et al, 1987) ie. those of most interest in determining functional significance.

Many of the problems relating to anatomical assessment occur because of the limitation of arteriography in reconstructing a three-dimensional lesion. Thus, the orientation of the vessel to the X-ray planes, stenoses at curvatures of the native vessel and asymmetrical narrowing lead to inaccuracy. Because the effective resistance at the site of the stenosis is proportional to the fourth power of the radius, small changes in radius beyond the resolution of arteriographic assessment may cause larger changes in resistance, particularly in more severe stenoses. Problems also arise when describing

the stenosis as a percentage of normal as many adjacent "normal" segments are affected by diffuse disease leading to an underestimation of stenosis severity. The use of intravascular ultrasound to document early atherosclerotic changes may indicate such disease in adjacent segments, but the problem still remains of predicting functional significance of lesions. There is a complex non-linear relationship between pressure gradient across a stenosis and flow through the stenosis which becomes accentuated with the increase in the severity of the stenosis. Because of this, the effective resistance of even a rigid stenosis (without a dynamic component) increases with an increase in myocardial oxygen demand ie. flow requirement.

Minimum luminal diameter is an absolute measurement but does not take into account the large variability in coronary artery diameter in normal subjects. Moreover, the eccentricity and irregularity of a lesion will determine the transtenotic pressure drop for a given luminal diameter due to flow separation and shear stress (Sibley et al, 1986; Wilson et al, 1987), causing much variability for the same absolute diameter. Estimates of the minimal cross-sectional area are complicated by uncertainty over the predicted reference area at the same point in a non-diseased artery, particularly in eccentric lesions.

These problems with conventional arteriography are compounded by the variables which, with epicardial coronary diameter, determine myocardial perfusion such as mean aortic pressure (perfusion pressure), venous pressure, collateral blood flow, resistive vessel function in the distal vascular bed and intra-ventricular wall stress (Kirkeeide et al, 1986). These variables assume even greater importance in the presence of a significant epicardial stenosis with progressive vasodilatation of the the vascular bed distal to the obstruction (Klocke, 1987).

2.3 Maximal Vasodilatation and the Coronary Vasodilator Reserve

The coronary flow reserve (or the coronary vasodilator reserve) has been proposed as an objective measurement of the vasodilatory capacity of the coronary resistive vessels, which regulate myocardial perfusion modulated by neural and metabolic influences. This was defined as the ratio of maximal coronary blood flow to basal coronary blood flow, ideally for a given perfusion pressure, by Gould in 1974 (Gould et al, 1974). Its validity has been confirmed and applied using different techniques such as coronary sinus thermodilution (Ganz et al, 1971), Doppler catheterisation (Wilson et al, 1985), and positron emission tomography (Bergmann et al, 1985) using a pharmacological stress such as intravenous dipyridamole or intracoronary papaverine.

Coronary vasodilator reserve has been widely used as a physiological measurement of the severity of a coronary artery stenosis, including all of its geometric characteristics (Kirkeeide et al, 1986). The ultimate effect of a coronary stenosis depends on the degree to which the increased impedance to flow is compensated for by vasodilatation at the level of the resistive vessels - the "reserve". Thus, the coronary vasodilator reserve may be seen in terms of autoregulation: the ability of the coronary vascular bed to maintain coronary flow at a constant level in the presence of a potential decrease in coronary perfusion pressure at a constant myocardial oxygen demand (Klocke, 1987) (Figure 2.1).

The coronary vasodilator reserve has a pharmacological maximum of around 5-6 times in man under conditions of a normal aortic pressure (Gould et al, 1990). It has been estimated from extensive animal studies that with the development of a 40% diameter stenosis, the coronary vasodilator reserve starts to diminish such that with a 85% diameter stenosis, the reserve would be "exhausted" and basal coronary flow

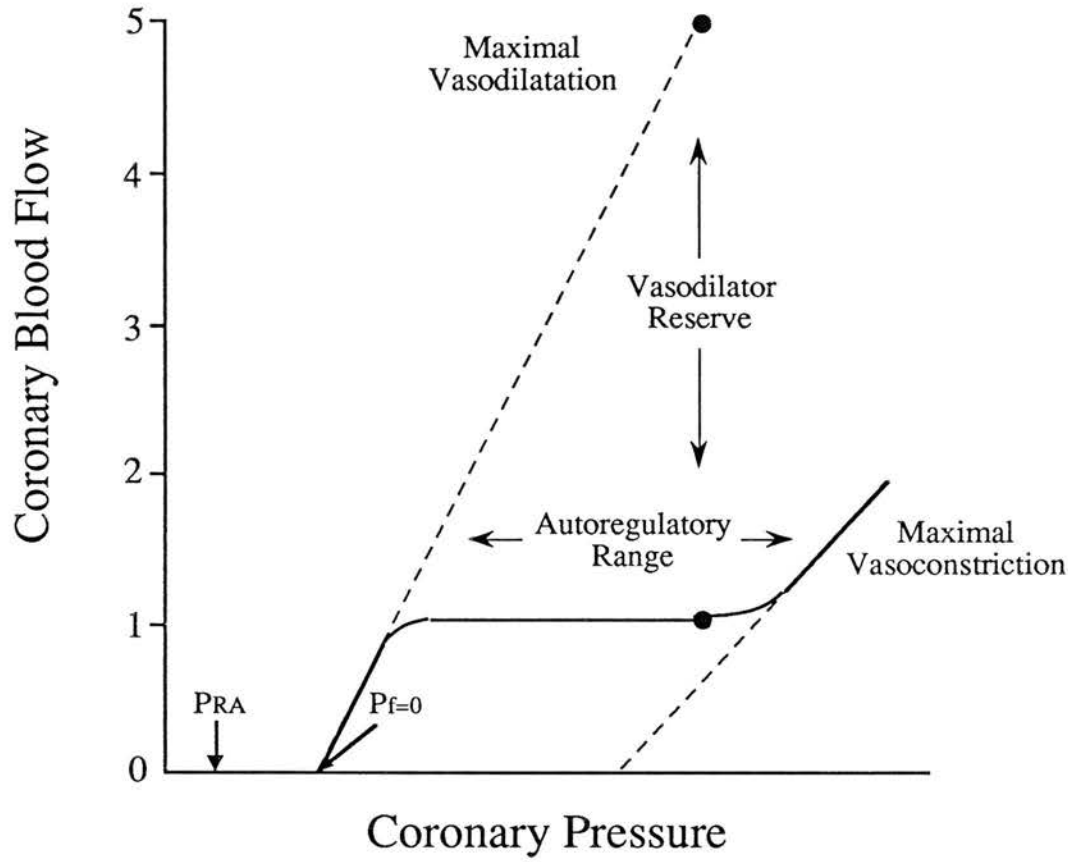


Figure 2.1. Steady-state relationship between coronary flow and coronary arterial pressure in the left ventricle. The solid line describes the normal relationship. At a constant level of myocardial metabolic demand, coronary blood flow is maintained constant over a wide range of coronary perfusion pressure, between the boundaries of maximal vasodilatation and vasoconstriction. The black circles represent the basal state and maximal blood coronary flow under normal conditions, giving a coronary vasodilator reserve of 5.0. P_{RA} = right atrial pressure, $P_{f=0}$ = pressure at zero flow (the back pressure opposing coronary flow). (Modified from Klocke, 1987)

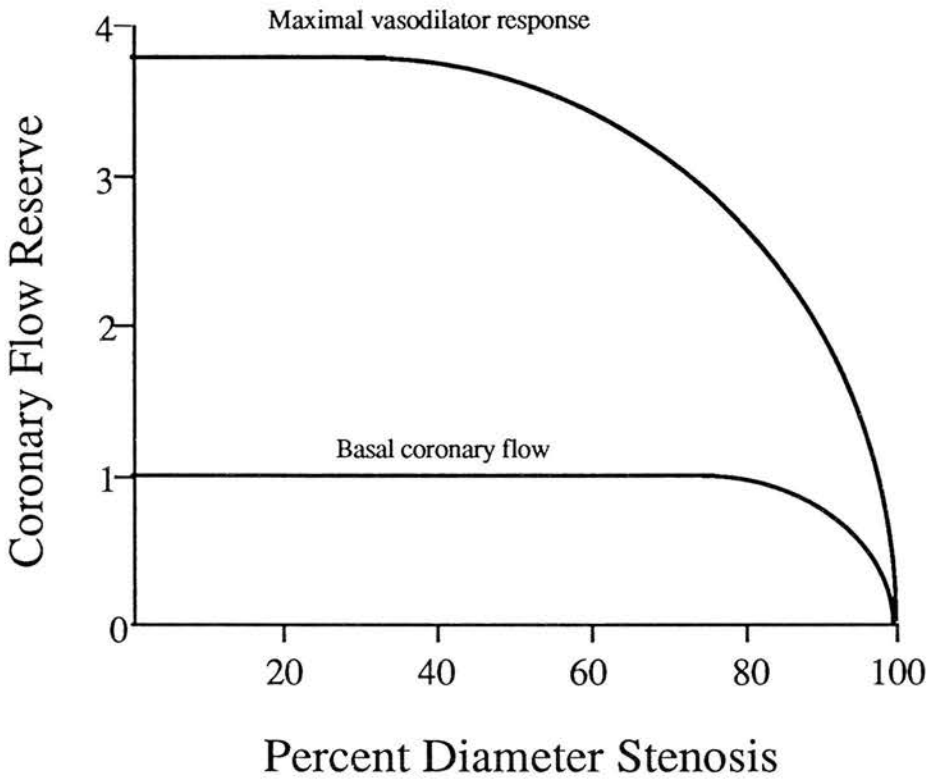


Figure 2.2. The relationship between basal coronary blood flow and maximal coronary blood flow in dogs. With progressive acute epicardial coronary stenosis, basal flow did not change until the coronary stenosis diameter exceeds 80% stenosis. Maximal coronary blood flow decreases from the development of a 50% stenosis (Modified from Gould et al, 1974).

start to diminish, ie. the point at which autoregulatory vasodilatation is maximal (Gould et al, 1974).

The coronary vasodilator reserve, whether used to describe the functional significance of an epicardial stenosis or used as an objective measure of the vasodilatory capacity of a myocardial region, may be altered by many different factors. When used to measure functional stenosis severity, because of the non-linear relationship between trans-stenotic pressure gradient and stenosis severity, a progressive non-linear reduction in vasodilator reserve is seen (Figure 2.2). There are three variables which need to be taken into further consideration when measuring the coronary vasodilator reserve at a given point in time; i) the coronary perfusion pressure, ii) the basal flow, and iii) the pressure-flow relationship during maximal vasodilatation.

i) Modest changes in aortic pressure can have a significant effect on maximal flow because of the steepness of the pressure-flow relationship during maximal vasodilatation. Alternatively, small dynamic changes at the site of a severe stenosis (or passive collapse of the stenosis due to distal vaodilatation) leading to a reduction in perfusion pressure may reduce reserve to a significant degree.

ii) Basal flow varies directly with myocardial oxygen demand (largely dependent on heart rate, contractility and myocardial wall tension), and as the denominator of coronary vasodilator reserve, a small increase in basal flow may reduce the derived value of vasodilator reserve accordingly. The control of basal coronary flow is determined by coronary autoregulation ie. pressure-dependent changes in vascular resistance, and tissue metabolism (Chapter 1, Section 1.4) (Figure 2.1).

iii) The pressure-flow relationship at maximal dilatation may be altered by several factors. Chronic hypertrophy may reduce the slope of this relationship considerably (Figure 2.3), such that a 30% increase in ventricular mass may lead to a 60% reduction in vasodilator reserve (Klocke, 1987). Haemodynamic parameters such as an increase

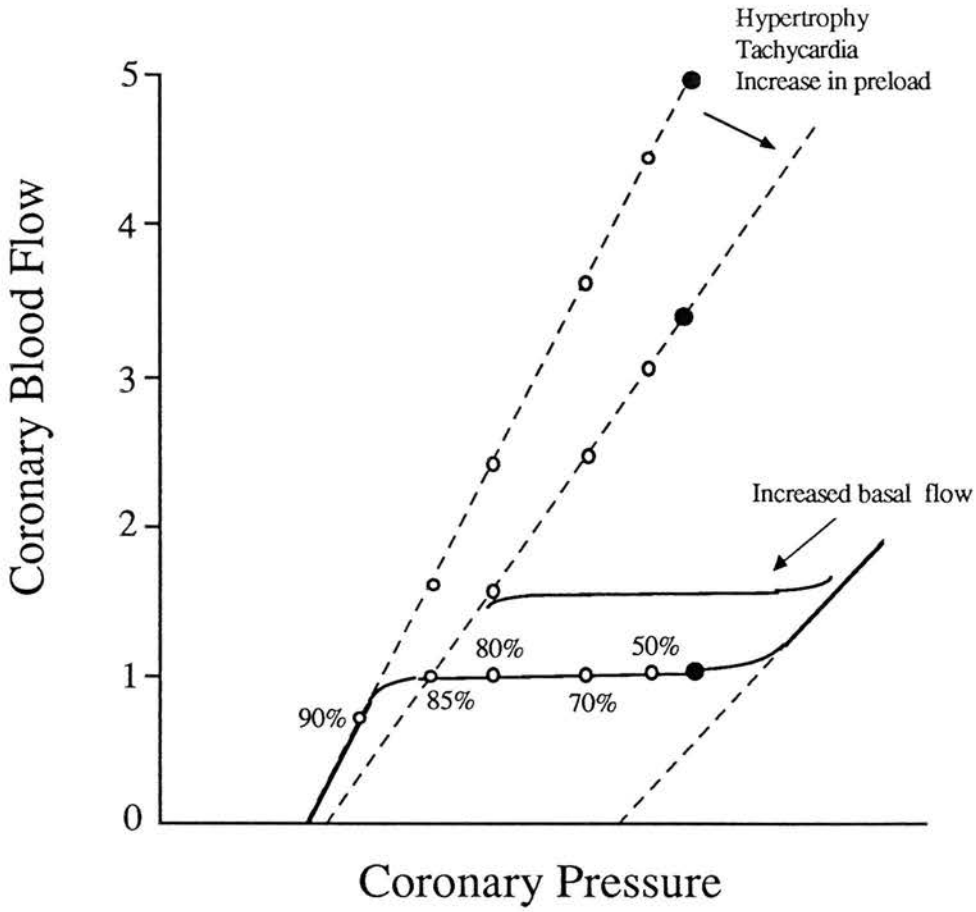


Figure 2.3. Mechanisms whereby the coronary vasodilator reserve may be altered. The basal coronary flow may increase with an increase in myocardial metabolic demand. Maximal vasodilatation may be reduced by an increase in heart rate or contractility, increased blood viscosity, and by resistive vessel dysfunction (in this case from 5.0 to just over 3.0). With hypertrophy, if coronary blood flow is measured as total flow ($ml \cdot min^{-1}$), then maximal vasodilatation may be approximate to control values but with an increase in basal flow because of the increase in muscle mass. If measured as flow per unit mass ($ml \cdot min^{-1} \cdot g^{-1}$) as depicted in the figure, then maximal vasodilatation is reduced and basal flow per unit mass the same as controls (Hoffman, 1987). With a reduction in perfusion pressure, usually caused by epicardial disease, a third variable is introduced. Coronary artery stenoses are the open circles expressed as % diameter stenoses. The horizontal distance along the autoregulatory range from control to stenosis is the trans-stenotic pressure gradient, which has a non-linear relationship with diameter stenosis. Thus, resistive vessel dysfunction due to epicardial disease may compound an already compromised vasodilator reserve. (Modified from Klocke, 1987).

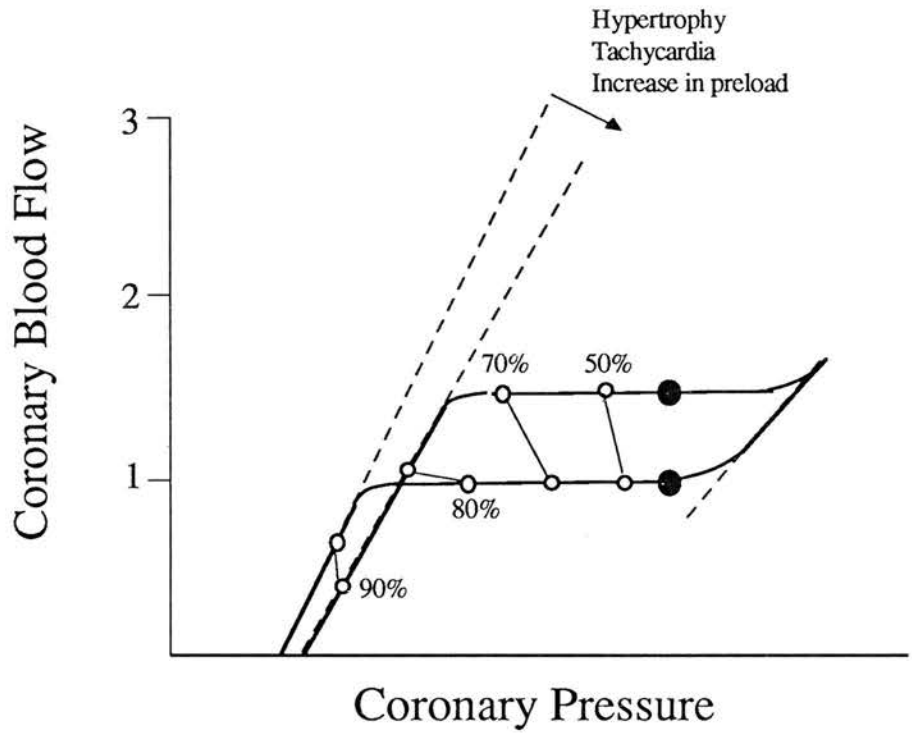


Figure 2.4. The presence of coronary artery disease adds to the complexity of the coronary vasodilator reserve as a concept. Increasing myocardial oxygen demand eg. with atrial pacing increases basal flow vertically in control subjects for a given perfusion pressure. With epicardial disease, the increase in flow leads to an increased pressure gradient across the lesion, as trans-stenotic gradients vary with flow (as well as with the degree of stenosis). With greater stenosis severity, the increase in basal flow is progressively limited, such that in the example, there is little increase in flow with an 80% stenosis ie. "exhaustion of reserve". With a 90% stenosis, flow is reduced. This is caused by the shift in the lowermost part of the pressure-flow relationship to the right. An increased heart rate does this by reducing diastolic filling time and increasing time-averaged compressive forces, the latter also augmented by increased contractility and hypertrophy. Increased preload increases $P_{f=0}$ and P_{RA}. (Modified from Klocke, 1987).

in contractility, preload, or heart rate may reduce maximal dilatation. An increase in left ventricular end-diastolic pressure (LVEDP) reduces maximal vasodilatation, and at low coronary perfusion pressures, this effect is accentuated such that at a perfusion pressure of 30 mmHg (= 85% stenosis) an elevation of LVEDP from 10 to 15 mmHg, would result in a 30% reduction in maximal flow. Tachycardia may reduce maximal dilatation by reducing diastolic filling time, notwithstanding the increase in myocardial oxygen demand and thus basal flow. It has also been shown that the effect of heart rate on maximal dilatation is greatest in the subendocardial layers because of the direct compressive effect of systolic contraction (Bache & Cobb, 1977). Other factors affecting coronary vasodilator reserve through an effect on maximal vasodilatation are blood viscosity and the extent of collateral flow which is difficult to quantify but which may be recruited by pharmacological agents (Klocke, 1987).

Coronary vasodilator reserve remains highly reproducible in the same subjects studied several months apart using intracoronary Doppler catheterisation (Section 2.4.2.4), with any differences between study related to changes in heart rate rather than mean arterial pressure (McGinn et al, 1990). In one study investigating the haemodynamic determinants of the coronary vasodilator reserve, atrial pacing led to a rate-related increase in basal flow but with no reduction in maximal flow at a rate of 120 beats·min^{-1}. A similar increase in basal flow was seen with volume expansion (an increase in preload), but with no effect on maximal flow. Increasing mean arterial pressure with handgrip caused a proportionate rise in basal and vasodilator flow (McGinn et al, 1990) and a maintenance or the coronary vasodilator reserve. This confirms the importance of interpreting coronary vasodilator reserve measurements taking into account the haemodynamic conditions at the time of study.

It is often assumed in clinical studies that the coronary vasodilator reserve is a globally homogeneous measurement with regional differences which occur perhaps as a consequence of epicardial disease or altered myocardial function. Animal studies

have suggested that there is marked spatial heterogeneity between different small regions of myocardium both under basal at maximal conditions (Austin et al, 1990). Regional coronary vasodilator reserve to adenosine may vary from 1.75 to 21.9 with no relationship between basal and maximal flow. However, in this study using microspheres, basal coronary flow and reserve appeared to be locally continuous perhaps defining functional zones of vascular control and vulnerability to ischaemia (Austin et al, 1990). With improvement in techniques used to measure myocardial perfusion in clinical studies, these observations may have implications in determining the control of regional microvascular function.

2.3.1. The Absolute and Relative Coronary Flow Reserve

Conventionally, with the development of proximal coronary disease to a significant degree, coronary resistance would be determined by the geometry of the lesion with a compensatory vasodilatation in the resistive vessels to maintain basal myocardial perfusion and the increased perfusion in response to increased myocardial demand (Gould et al, 1974). However, percent diameter stenosis is poorly related to the functional severity of the coronary obstruction at quantitative angiography (Demer et al, 1989) or the coronary vasodilator reserve at Doppler catheterisation (White et al, 1984). Cardiac workload (and thus myocardial oxygen consumption) may be affected by changes in aortic pressure (coronary perfusion pressure) and heart rate leading to changes in the basal coronary flow and ultimately maximal vasodilatation (Gould et al, 1990). Thus, the absolute coronary vasodilator reserve may be affected by these determinants independent of stenosis geometry. This may diminish the value derived under varying physiological conditions and diminish valid comparisons between different patients. This has lead to the concept of the relative coronary vasodilator reserve which is the maximal coronary flow in the index artery/region divided by the maximal coronary flow in the control artery/region. As this ratio cancels out the effect

of the prevailing physiological conditions on coronary flow in both regions, this relative value reflects geometric stenosis severity, as long as there is maximal vasodilatation in the control region.

The ability to measure absolute and relative coronary vasodilator reserve is one of the major advantages of positron emission tomography. The quantitation of myocardial blood flow at basal and at maximal vasodilatation in the region of interest allows calculation of the absolute vasodilator reserve and the comparison with a control region determines the relative vasodilator reserve, thus providing complementary information in the same study.

Until the development of positron emission tomography, all methods used to measure basal and maximal coronary blood flow, and thus the coronary vasodilator response, were invasive thus interfering with true basal flow either through instrumentation or injection of contrast, thus leading to an underestimation of this ratio. It has also been argued that the coronary vasodilator response may appear diminished due an increased basal flow in certain conditions such as left ventricular hypertrophy or valvular disease where it is argued that "vascular function" is normal (Hoffman, 1987).

This argument has led to the proposal that it is maximal myocardial blood flow alone that determines the functional status of the coronary circulation (Pijls et al, 1990), and that it is independent of other confounding variables. This view however, ignores the fact that the earliest sign of resistive vessel dysfunction may be a change in basal myocardial blood flow per se, before maximal flow is affected.

2.3.2. Pharmacological Vasodilatation

Many different stimuli have been used to achieve maximal coronary vasodilatation in order to assess the coronary vasodilator reserve. Using atrial pacing (Holmberg & Varnasukas, 1971), and intracoronary hyperosmolar contrast media (Bassan et al,

1975) or intravenous isoprenaline (Horwitz et al, 1974), values of between 2-2.5 have been achieved which is well below maximal vasodilatation (Gould et al, 1990). With maximal exercise, coronary blood flow increases from 2-3.9 times control values (with a reduction in coronary resistance to 36% of basal) (Holmberg et al, 1971). However, such an increase in flow may not be maximal as it is possible to increase flow by another 35% in dogs after maximal exercise with dipyridamole (Barnard et al, 1979). This is evidence of the dissociation between vasodilatation due to an increase in myocardial demand and that due to a pharmacological stimulus.

The maximal coronary flow possible is probably the hyperaemic flow seen after a 20 second occlusion of an epicardial coronary artery at surgery which leads a coronary vasodilator reserve of 5.5-6.3 (Marcus et al, 1981). The coronary vasodilator reserves of 3.0-5.0 seen following pharmacological dilatation with intravenous dipyridamole (Brown et al, 1981), with the addition of isometric handgrip, give the most easily obtainable maximal values in the non-invasive environment. The most widely used pharmacological vasodilators are intravenous dipyridamole and adenosine, and intracoronary papaverine.

1. Dipyridamole

Dipyridamole is an adenosine deaminase inhibitor and by blocking uptake of adenosine produced locally by red blood cells, platelets and endothelium, potentiates the activity of adenosine. Adenosine then acts directly on vascular smooth muscle on the P_1-purinergic receptor coupled to the adenylate cyclase enzyme. Systemic effects include a fall in arterial blood pressure and an increase in heart rate. Distal to a coronary artery stenosis, blood redistributes from the endocardial to the epicardial layers - transmural coronary "steal" associated with a fall in distal perfusion pressure.

It has been argued that dipyridamole induces less than maximal dilatation in as many as 25% of normal subjects and thus elicits a lesser coronary vasodilator reserve

than intracoronary papaverine (3.7 vs. 4.4 respectively) (Rossen et al, 1991). For this reason, many advocate the use of isometric handgrip to increase systemic vascular resistance and thus increase aortic pressure and coronary perfusion pressure (Brown et al, 1981; Rossen et al, 1989). Others advocate using a dose higher than the standard (0.56 $mg \cdot kg^{-1}$ over 4 minutes) by adding another 0.28 $mg \cdot kg^{-1}$ (Picano et al, 1991). There is also a variability in the time to peak effect of dipyridamole in different patients (Wilson et al, 1985).

Another limitation with dipyridamole is that it has a longer duration of effect (over 20 minutes) than other vasodilators often making quick serial studies during the same procedure impossible, although if only being used once eg. at PET scanning, this is not such a problem. Dipyridamole is well tolerated as a vasodilator although some patients suffer chest pain, headache and dizziness, it remains the most widely used vasodilator for perfusion imaging.

2. Adenosine

Adenosine is usually given at a dose of 140 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and is said to elicit a similar vasodilator response as intracoronary papaverine (Wilson et al, 1990). Adenosine and dipyridamole produce similar haemodynamic effects when given by intravenous infusion. It has been argued that adenosine gives maximal vasodilatation with less individual variability in response compared to dipyridamole (Wilson et al, 1990). However, more side effects are reported with adenosine requiring intervention, 9% in one study (Nishimura et al, 1992), and there is the increased tendency to develop atrio-ventricular node inhibition in a small number of patients, albeit transient and asymptomatic (Nishimura et al, 1992). It has been proposed that the effect of adenosine is consistent and near-maximal (Wilson et al, 1990), perhaps suggesting an advantage over dipyridamole. However, in one prospective study using PET, no difference in the vasodilator response to either agent was found (4.4±0.9

$ml \cdot min^{-1} \cdot g^{-1}$ with adenosine vs. 4.3±1.3 $ml \cdot min^{-1} \cdot g^{-1}$ with dipyridamole), with a similar range in the individual coronary vasodilator reserve (2.0-8.4 for adenosine vs. 1.5 to 5.8 for dipyridamole) (Chan et al, 1992). To date, no one study has convincingly shown that adenosine is superior to dipyridamole as an intravenous coronary vasodilator stimulus.

3. Papaverine

Papaverine is an opiate derivative which is produces direct relaxation of arteriolar smooth muscle. It has become a widely used vasodilator for measurement of the coronary vasodilator response using Doppler catheterisation when given intracoronary. This is because it produces rapid maximal vasodilatation (at 15 seconds) returning to 10% of basal values within 128 seconds (Wilson et al, 1985), compared to over 4 minutes with dipyridamole which has a duration of action of over 30 minutes. Although maximal vasodilatation may be dose-dependent (ranging from boluses of 6-12 mg), the hyperaemic period is sufficiently brief to allow repeat measurements. Given intracoronary, there are only minimal systemic haemodynamic effects (Wilson et al, 1985). However, in dog studies, although producing equivalent coronary vasodilatation to intracoronary adenosine, ST segment depression, QT interval prolongation (and occasional ventricular fibrillation) and lactate production are found with intracoronary papaverine (Christensen et al, 1991). Furthermore, papaverine also causes a bizarre double contraction-relaxation effect perhaps by an inhibitory effect on oxidative phosphorylation or on calcium uptake (Christensen et al, 1991). For these reasons, current practice has moved towards the use of intravenous dipyridamole and intracoronary adenosine.

2.4 Methods of Measuring Myocardial Blood Flow

It is important to make an early distinction between myocardial blood flow, which is the perfusion of a finite volume (or mass) of myocardium by a volume of blood per unit time, and coronary blood flow, which is the volume of blood per unit time flowing down an epicardial coronary artery. Although in normal subjects the two may be approximate, it is important to draw a distinction in disease states as with changes in myocardial mass, the two may become dissociated, particularly as the determinants of proximal flow and distal perfusion may have changed.

Flow may be measured with three different methods in animal models which are more accurate than what is generally practicable in human subjects.

2.4.1. Laboratory Methods

2.4.1.1. Electromagnetic Flow Measurement

This method uses the principle of induction where blood acts as a moving conductor in a magnetic field placed perpendicular to the direction of flow, producing an induced voltage proportional to the amount of flow. This method is accurate and integrates flow across the whole vessel but the probes are large and require to be implanted into the wall of the vessel. Calibration is also difficult with this technique, particularly when used intra-operatively placed on vein bypass grafts because of poor vessel contact and changes in distending pressure. This method is no longer used in clinical practice.

2.4.1.2. Epicardial Ultrasonic Flow Velocity Measurement

Reflection of ultrasound waves emitted from a piezo-electric crystal from moving red blood cells occur at a given frequency according to the Doppler equation:

$$f_d = 2f_0 \cdot \frac{v \cos a}{c}$$

where f_d is the frequency shift, f_0 the frequency of emitted waves, v the velocity of the red blood cells, a the angle of the ultrasound waves relative to the direction of flow and c the velocity of ultrasound in blood (c. 1,500 $m \cdot s^{-1}$). This method is used in exposed coronary arteries (Marcus et al, 1981) usually as a circular probe. The diameter of the vessel should be considered to allow calculation of absolute coronary flow. Flow at one point is determined at the centre of the flow wave and assumes laminar flow; thus the calculated flow is proportional to rather than equal to actual flow.

These two methods correlate very well with each other and have the advantage of allowing a high frequency response and unlimited measurements.

2.4.1.3. Microspheres

These radio-labelled particles are usually injected into left atrium and are of sufficient size to become trapped in the coronary capillary network, subsequently measured using a gamma counter after animal sacrifice. Myocardial flow is expressed as a ratio to flow taken from a peripheral arterial sample at the same time as injection (Buckberg et al, 1971). This method allows quantitation of transmural myocardial perfusion but is limited by a low frequency response, loss of microspheres, few separate measurements allowable, and radiation exposure.

2.4.2. Clinical Methods

Methods for measuring myocardial and coronary blood flow which are applicable to man are less accurate, generally not widely available (Marcus et al, 1987), and may put the patient at a small but additional risk because of the invasive nature of the method in question. However, in clinical terms, the major derivative is often what

relative changes there have been in flow or perfusion, rather than absolute measurements of coronary flow.

2.4.2.1. Coronary Sinus Thermodilution

The principle of thermodilution to measure cardiac output was first developed using a flotation balloon catheter in the pulmonary artery by Swan and Ganz, and has also been applied to the measurement of coronary venous blood flow by insertion of a thermodilution catheter into the coronary sinus (Ganz et al, 1971). Although this method involves only right heart catheterisation, coronary flow is assessed only in the territory of the left anterior descending artery. Regional left ventricular flow measurements are confined to crude separation of great cardiac (anterior) vein flow (anterior wall and septum) and perfusion draining to the distal coronary sinus. Even then, the venous drainage of left ventricle is variable with substantial venous collaterals, meaning in effect that drainage alone from the great cardiac vein is an accurate representation of left anterior descending (LAD) artery flow under basal conditions (Rossen et al, 1992). Following LAD occlusion and hyperaemia, great cardiac vein flow underestimated both the decrease and the increase in flow (Cohen et al, 1988). A steady state is essential as several seconds are required to measure flow and thus phasic flow cannot be measured. Inaccuracies arise from inadequate mixing of indicator and blood along with coronary sinus reflux. In contrast to in vitro studies, the technique has been poorly validated in the in vivo situation (Mathey et al, 1978; Pepine et al, 1978). Furthermore, it has been demonstrated that coronary sinus thermodilution consistently underestimates coronary vasodilator reserve compared to Doppler catheterisation in the same patients (Rossen et al, 1992).

Substantial alterations in flow of the order of 25-30% are required for accurate measurement, and in coronary artery disease, reliability markedly diminishes (Marcus

et al, 1987). For this reason, the technique should only be used in patients with normal coronary arteries where large changes in coronary flow are expected.

2.4.2.2. Gas Clearance Methods

The principle of this method is similar to that of coronary sinus thermodilution. Inert gases may be given by inhalation (non-radioactive helium/argon) or intracoronary injection (^{133}Xe) and an arteriovenous difference occurs across the coronary vascular bed due to myocardial extraction. Myocardial perfusion may be calculated from the uptake and washout of gas with the measurement of coronary arterial and venous samples (Marcus et al, 1987), but similar limitations to the thermodilution method exist such as the inability to measure rapid changes in flow and the problems of variable venous drainage. Spatial resolution has been improved with the use of ^{133}Xe although because of its high fat solubility, only a limited number of studies can be done. Furthermore, accurate timing of any intervention is required because of the long time constant of the method and accuracy is reduced at higher flow rates (Maseri et al, 1977). Consequently, these problems have limited the widespread use of this method.

2.4.2.3. Videodensitometry

The changes of contrast density in the myocardium after the intracoronary power-injection of contrast may be measured and used to calculate coronary flow using the indicator dilution theory. This principle was first described in in saphenous vein grafts by Rutishauser and co-workers in 1967 who used the technique with respect to flow (Rutishauser et al, 1967), but avoided the need to know the exact amount of indicator injected into the graft, as was necessary for previous methods in the systemic circulation. In this method, flow may be calculated from the mean transit time of a contrast bolus through a measured length and diameter of an arterial segment (Rutishauser et al, 1967). However, when applied to the coronary circulation, the

method was less accurate because of the complex three-dimensional course of native arteries with multiple branch points.

Using ECG-triggered subtraction imaging to enhance visualisation of myocardial contrast passage, myocardial flow in a region of interest may be calculated from the time-density curve. Analysis of the changes in contrast density and description of this curve to calculate the mean transit time using digital subtraction angiography has improved the spatial resolution of this technique (Vogel et al, 1984). In one of the first validation studies, Hodgson et al compared contrast density/appearance time ratios with vasodilator reserve measured by an electromagnetic flowmeter and demonstrated a correlation of R=0.92 (Hodgson et al, 1985). However, 95% confidence limits ranged from 1.2 to 2.7 at a vasodilator reserve of 2.0, and a similar spread has been reported by others (Cusma et al, 1987)

The problems with this method are that the contrast agent itself induces a hyperaemic response (a biphasic effect), even when using non-ionic iso-osmolar agents such as Iohexol-140. This may be worsened by reflux of contrast into the aorta and distribution into different branches of the coronary vascular bed. This makes the accurate measurement of basal myocardial blood flow almost impossible. Contrast media is a poor vasodilator with a variable dose-response relationship between patients, and the peak flow effect may be missed; the use of intracoronary papaverine as a vasodilator has improved the accuracy of the technique (Graham et al, 1991). The method also requires estimation of the vascular volume between injection site and myocardial region of interest which changes with alterations in arteriolar resistance, and thus volume of the resistive vascular bed (Gould et al, 1985; White et al, 1984). Furthermore, stability of the image for up to 20 seconds ie. absolute breath-holding by the patient, is essential to allow measurement of the mean transit time from a good quality time-density curve.

Although many of these limitation may be overcome by mathematical assumptions and rigourous technique including atrial pacing, only accurate measurement of maximal myocardial blood flow may be achieved (Pijls et al, 1990). As will be discussed later in this thesis, alterations in basal myocardial blood flow are a common pathophysiological feature of altered resistive vessel function, and it is for this reason that positron emission tomography remains the best non-invasive method for the absolute quantitation of basal and maximal myocardial blood flow.

2.4.2.4. Doppler Catheterisation

The use of an intracoronary Doppler catheter to measure coronary blood flow velocity was developed at the University of Iowa in the mid-1980s. The catheter consists of a 20 MHz piezo-crystal mounted at the top of a steerable 3F gauge catheter which may be inserted into a coronary artery over an angioplasty guide wire (Wilson et al, 1985; Sibley et al, 1986). This allows selective and repeated measurement of coronary flow, and like epicardial probes, these catheters have an excellent frequency response. They have been well validated against other methods (Marcus et al, 1981; Wilson et al, 1985). However, because of the invasive nature of the procedure, there is a small risk of injury to the coronary artery under study, a risk which is compounded by the relatively unstable free-floating tip which can lead to a variable quality of the recordings. The original method using the suction Doppler intraoperatively measured only changes in velocity although absolute velocity may be estimated from the frequency shift using the intracoronary catheter. With the Doppler technique, the normal coronary vasodilator reserve is 5.0 with no difference reported with gender, age or study vessel (Marcus, 1983b).

In the presence of epicardial coronary artery disease, the catheter tip may be inserted distal to the stenosis to achieve reliable measurements. However, this in itself can lead to increased obstruction at the site of the stenosis, with further disturbance of

laminar flow, in a situation where the distribution of velocities in a cross-sectional area is already unknown. Furthermore, with passage of the catheter into smaller vessel diameters (<2.5 mm^2), overestimation of the severity of the flow obstruction will also occur. The catheter tip may be placed proximal to the lesion (up to 5 mm) to give an estimate of flow at the point where there is a reduction in flow. However, given that changes is vessel diameter in this proximal segment may occur with vasodilatation, this diameter and thus the cross-sectional area of the so-called reference segment should be included in the estimation of coronary blood flow from coronary flow velocity. Some avoid this by pre-administration of nitroglycerin, but this obviates study of changes in vasomotor tone in the artery under investigation (Jost et al, 1990), and in general, only trivial changes in intracoronary diameter occur after administration of intracoronary papaverine (Wilson et al, 1986) or intravenous dipyridamole (Brown et al, 1981). Thus, intracoronary Doppler is widely used by groups studying the pathophysiological changes in coronary blood flow due to coronary artery disease.

Measurement of the coronary vasodilator reserve using the Doppler catheter correlates reasonably well with the stenosis severity whether described as percent diameter stenosis (R=0.82), percent area stenosis (R=0.85), or minimum cross-sectional area (R=0.79) determined from orthogonal planes (Wilson et al, 1985). Despite these reasonable correlations in a highly selected group, there was substantial scatter on individual vasodilator reserve values at any level of stenosis. This variability underlines the additional factors affecting coronary flow in the presence of an epicardial stenosis such as angiographically unapparent collateralisation and resistive vessel function distal to the stenosis.

There have been several developments with the principle of Doppler catheterisation such as the Judkins Doppler-tipped catheter which may be used for the measurement of coronary flow velocity in normal arteries at the right or left coronary ostia. Most recently, the guide wire Doppler has been developed which obviates the need for a

Doppler catheter and additional instrumentation (Doucette et al, 1992). Another advantage of this catheter is that coronary flow velocity may be measured both proximal and distal to a coronary stenosis without significant interference in the cross-sectional area of the epicardial stenosis providing it is not a severe lesion. To avoid intracoronary instrumentation, transoesophageal Doppler echocardiography has been developed to avoid this problem (Iliceto et al, 1991). The use of Doppler catheterisation with intravascular ultrasound should allow absolute quantitation of coronary blood flow in the future.

2.4.2.5. Three-Dimensional Imaging Techniques

These non-invasive techniques include ultrafast computed tomography (CT), magnetic resonance imaging and positron emission tomography (Chapter 3). The first two techniques involve the principle of the indicator dilution theory. However, true validation of ultrafast CT and MRI is in its early stages and both techniques remain predominately research methodologies. Ultrafast CT has been applied to the measurement of flow in bypass grafts using similar assumptions as were done with early densitometric techniques (Section 2.4.2.3), with the advantage of an intravenous injection of contrast (Rumberger et al, 1986).

None of the previous methods described can study coronary flow in specific transmural layers and stratify the extent of ischaemia from epicardium to endocardium. It is hoped that ultrafast CT and magnetic resonance imaging may provide this information in time. In experimental studies, contrast echocardiography may be used to define the transmural extent of myocardial infarction by measuring the delay in contrast enhancement (Kemper et al, 1986), although no absolute transmural quantitation of flow is possible with this technique. This will becomes possible with the development of advanced PET cameras with improved spatial resolution, as it is

currently possible to distinguish epicardial from endocardial flow in patients with ventricular hypertrophy (Camici et al, 1991c).

2.5 Summary

The coronary vasodilator reserve is an attractive concept and allows not only a functional assessment of epicardial stenosis severity but also defines the vasodilator responsiveness of a coronary vascular bed, and thus coronary resistive vessel function. It is important to separate from the coronary vasodilator reserve, the effects of other variables such as heart rate, loading conditions, contractility and hypertrophy. Several methodologies have been developed to measure coronary flow in the clinical environment, the best validated being intracoronary Doppler catheterisation and positron emission tomography. The next stage of development of these methods is their application to the study of cardiac pathophysiology where the contribution of altered resistive vessel function to changes in the coronary vasodilator reserve may be defined and therapies developed to modify such abnormalities.

CHAPTER 3. POSITRON EMISSION TOMOGRAPHY AND THE ASSESSMENT OF MYOCARDIAL FUNCTION

3.1 Introduction

Positron emission tomography (PET) is an imaging technique which uses the detection of radiation from relatively short-lived positron-emitting radioisotopes delivered in vivo labelled to compounds taken up from the vascular bed as part of normal cellular function. Localisation of such radiation is sufficiently accurate to allow data presentation of the object studied, with the intensity of each picture element proportional to the isotope concentration at that position. As positron-emitting isotopes can be generated from most elements of the periodic table, organic molecules may be labelled with radioisotopes such as carbon-11, nitrogen-13, and oxygen-15, without altering the chemical structure of the compound used. These isotopes have been applied to the study of (myocardial) tissue function in the normal and the disease state. In this chapter, the principles and theory behind PET imaging and its application to the non-invasive measurement of myocardial blood flow and metabolism are described.

3.2 Basic Principles

Positron emitting radioisotopes are proton-rich with an excess positive charge on the nucleus and may be generated by linear acceleration in a cyclotron, such as carbon-11 (half-life = 20.4 minutes), nitrogen-13 (half-life = 9.8 minutes) and oxygen-15 (half-life = 2.1 minutes), or from a generator, such as rubidium-82 (half-life = 78 seconds). Such radioisotopes have short half-lives which permit the use of radiation

dosages compared to conventional myocardial tracers such as thallium-201, and the option of the use of different radioisotopes in the same scan. However, because of their short half-lives, an on-site cyclotron is required for production.

The basic principle of physics on which positron emission tomography depends is that of annihilation radiation and coincidence detection of this event. Two means of decay exist for such nuclei: i) capture of an orbital electron by the nucleus, or ii) emission of a positive election (a positron) from the nucleus. After travelling a short distance, the positron (which is an anti-electron) collides with an electron in orbit and they annihilate to produce electromagnetic radiation. This so-called annihilation radiation of two gamma rays of 511keV are emitted at 180° to each other simultaneously and can be detected externally by taking advantage of this phenomenon (Phelps, 1977) (Figure 3.1). Coincidence detection of these two photons on opposite sides of the study object places the site of annihilation on a line connecting the two external detectors, which are linked themselves by a coincidence circuit. Thus, annihilation occurring outside the detector volume is electronically rejected as only one photon is detected.

To determine the three-dimensional isotope distribution, measurements must be taken from several directions. Thus, radiation detectors scan across an object to obtain a count rate profile for a certain slice through the object, and then repeat this for a number of angles around the cross-section defined by the slice. This allows reconstruction of an image from isotope concentrations. This reconstruction is dependent on the physics of positron decay, positron annihilation, gamma-ray interactions within the object, as well as the geometry and engineering of the system. There are several methods for reconstructing the data to minimize error from assumptions made from count detection, which is outwith the scope of this chapter (Hoffman & Phelps, 1986). Suffice it to say, PET systems are designed to maximise

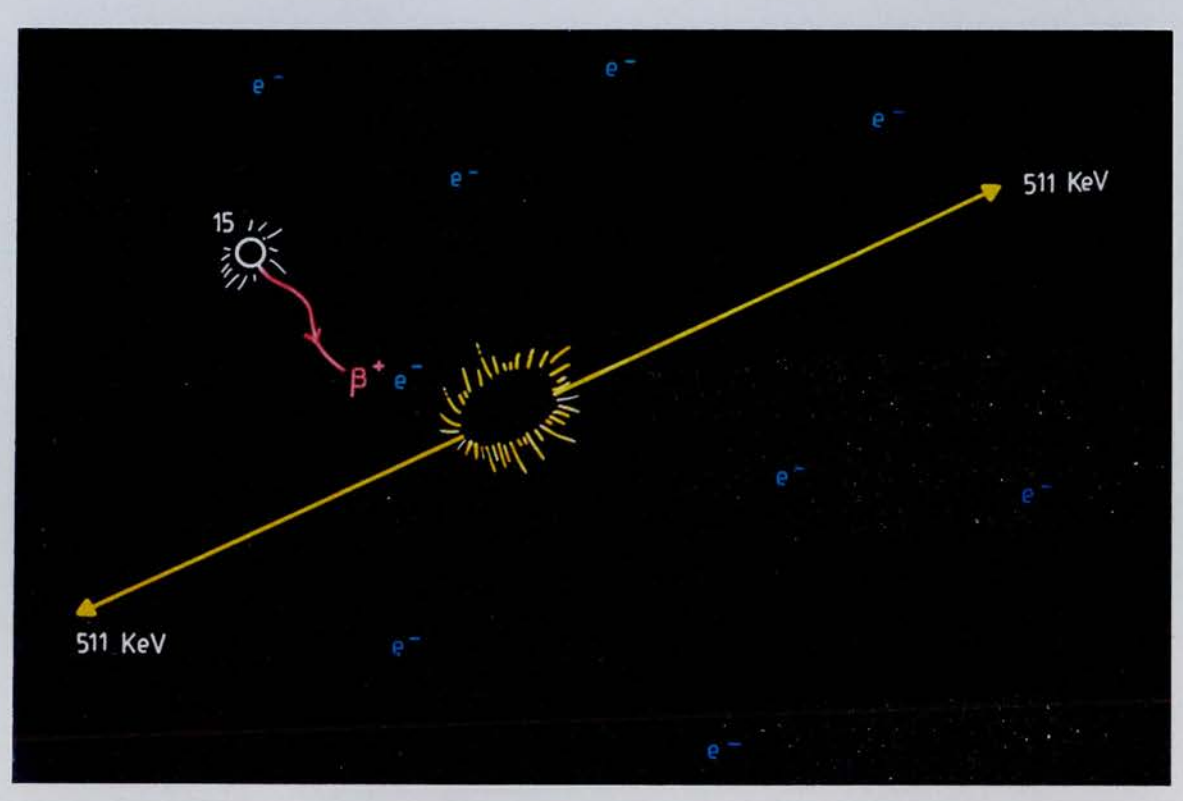


Figure 3.1. Emission of a positron (β^+) from the nucleus of a molecule of 15-oxygen. Collision with an electron (e^-) leads to annihilation radiation of two 511keV photons of gamma radiation in always at 180° to each other.

the detection of coincidence events (unscattered photons) and minimize background events from scattered photons and high count rates from outside the filed of view.

Two factors serve to limit the accuracy of annihilation coincidence detection to identify the site of decay of the positron emitting isotope: the positron range effect and the non-colinearity effect. The positron range effect describes the fact that the distance a positron travels before annihilation depends on its energy which, in turn, depends on the parent nucleus eg. positrons from ^{18}F nuclei have lower energy than those from ^{82}Rb nuclei. Positrons with lower energy combine with electrons at a smaller distance from the nucleus, allowing better localisation of decay. Non-colinearity occurs because of the momentum of the positron as it annihilates with the electron, causing one of the gamma photons to fall outside the detection volume of the block detectors. These factors account for the limit to the resolution of the current scanners.

3.3 The Block Detector Positron Emission Tomograph

Modern PET systems are designed with regular polygonal or circular rings of detectors to maximize the detection efficiency per slice. These are isolated by lead or tungsten as shielding for off-plane activity, but are at the vertex of a fan beam of sensitivity across the field of view, thus increasing sensitivity. The advantage of the circular sampling system is that there is no longer a need for the detector banks (hexagonal or octogonal array of blocks) to rotate to compensate for under-sampling. High density detector materials have reduced the need for movement of the detector system, such as a "wobble" about the centre of the field of view to increase sampling, and thus improve resolution and reduce artefact.

The scanner used in these studies was the ECAT 931/08-12 (Siemens-CTI Inc. Knoxville, Tennessee, U.S.A.) (Spinks et al, 1988) As described above, this scanner uses the principle of annihilation coincidence detection but has 8 rings of detectors each with 512 detector elements made of bismuth germanate. The detector elements are coupled to photomultiplier tubes in the ratio of 32 to 4 (Figure 3.2), and localisation of the detector in which the count is registered occurs through analysis of the peaks from all 4 photomultiplier tubes. Thus, any one detector is in coincidence with other detectors in the same and adjacent rings. There is an axial field of view of 10.8 cm consisting of 15 planar slices, 8 direct planes and 7 cross planes. Thus data acquisition occurs in direct planes between detector pairs in the the same ring, and in cross planes between detector pairs in adjacent rings of detectors, giving better axial resolution in the latter case.

The spatial resolution of the PET camera is assessed by the accuracy in localising a radioactive point source the field of view of the camera (Figure 3.3). This is determined from the gaussian-shaped profile resulting from the measurement of a radioactive line source positioned axially in the camera. The width of the count profile at 50% of the peak count is a commonly used determinant of the spatial resolution. Using the ECAT 931/08-12, spatial resolution is 8.4 mm at full width half maximum (FWHM) at the centre of the field of view (Spinks et al, 1988).

3.4 Quantitation

The quantity actually measured in PET is the local tissue concentration of positron emitter. This quantity can be related to a physiological or metabolic process through the application of an appropriate mathematical model of the process. With a uniform response from the annihilation coincidence detection system and attenuation correction (see below), it becomes practical to do this with PET.

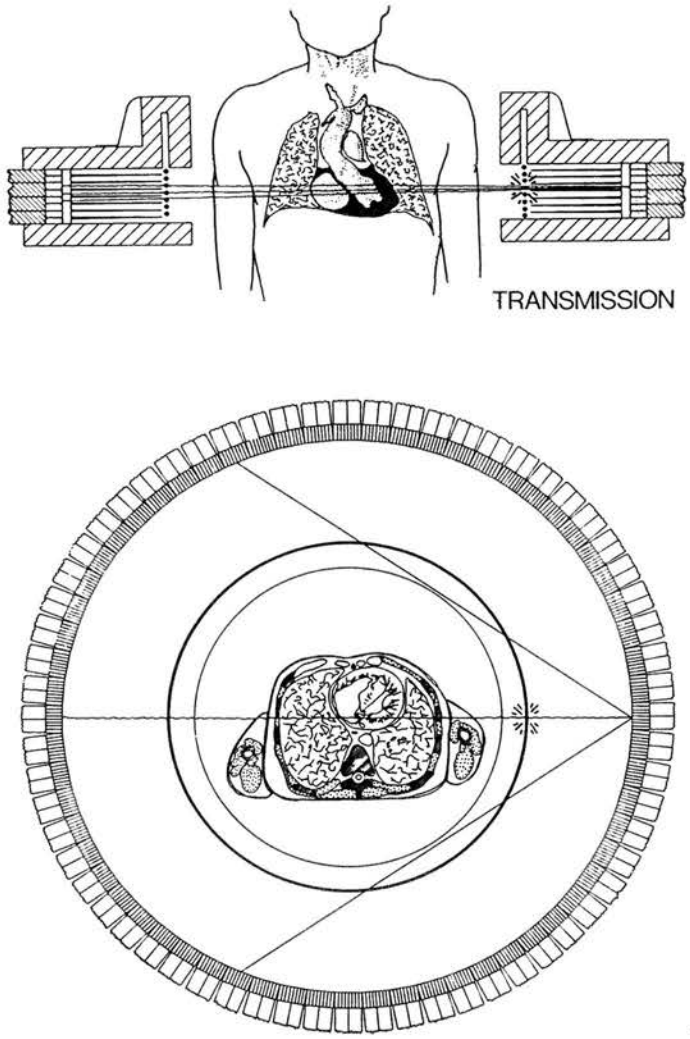


Figure 3.2. External irradiation of the subject with a ring source to generate a transmission scan. The patient is positioned transaxially at the centre of the ring source and the block detectors. The detector elements are coupled to photomultiplier tubes in the ratio of 4 to 1.

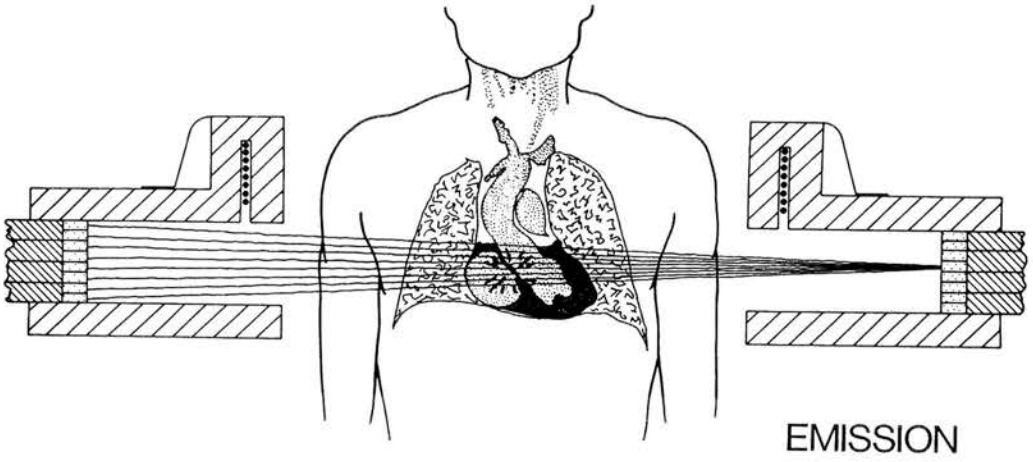


Figure 3.3. Gamma radiation from a positron emitting tracer in the myocardium. The block detectors are positioned transaxially around the patient, and should they detect the two annihilation photons simultaneously (in coincidence), then the original site of the annihilation event can be localised to the volume between the two detectors. Gamma photons outside this volume may only interact with only one of the detectors per annihilation. By electronically selecting coincidence events only, and rejecting all others, all true coincidence events can be localised accurately.

3.4.1. Calibration

The first step towards quantitation is accurate calibration of the system. This is done regularly by measurement of a positron emitter in a uniform source in a cylinder much larger than the spatial resolution of the tomograph. This is followed by measurement of activity in an aliquot of the solution with a radiation detector, such as a well counter. Every picture element (or pixel) in the generated image may then be scaled by this calibration factor to be equal to the activity at that location in the subject under study. The absolute activity is of less importance, as it is knowledge of the concentration in the blood and response of the PET system that determines quantitation. Thus, in cardiac studies, using $C^{15}O$ to produce [^{15}O]carboxyhaemoglobin in red blood cells, after coming to equilibrium, a region of interest in arterial blood such as left atrium or ventricle may be drawn on the image and venous samples taken and counted in a well counter. From this measurement, the response of a calibrated PET system can be calculated for 100% blood volume in the region of interest.

With $C^{15}O$, the dynamic equilibria existing among the delivery of the activity to the organ, the physiological washout of the activity of the organ, and the physical delay of a short-lived isotope allow the formulation of a mathematical model of the process that requires only one PET image and the amount of activity in several venous samples to measure the arterial input function. When measuring a more complex variables, the amount of activity measured in the organ depends on the kinetics of the metabolic process, the transport properties between the blood and the site of the process in the cell, and the amount and time course of the delivery of the activity to the organ.

Initially, direct arterial sampling was used to take a series of blood samples following delivery of the positron emitter to define the activity and shape of the arterial input function. However, the PET system may be used to measure the input function

directly by measuring the amount of activity in the left atrium or ventricle as a function of time thus relying only on venous sampling.

3.4.2. Image Reconstruction

The scanner collects coincidence data which are reconstructed into images quantitatively representing the radiotracer distribution in the field of view. This is done by using filtered back projection which produces such images, the algorithms for which run on dedicated array processors on a MicroVax II computer. This technique relies on several assumptions; i) transaxial resolution is constant throughout the field of view, ii) there is no absorption of gamma photons between coincident detector pairs, iii) all measured counts are true coincidences (independent of coincidences from scattered and random photons), and iv) there are no lost counts because of detector dead-time. The design of the current camera in use ensures that transaxial resolution is constant, and that both scattered/random coincidences and dead-time losses are corrected for. Absorption and attenuation of photons by structures in the field of view account for the main problem in determining accurate image reconstruction.

3.4.3. Attenuation Correction

Annihilation gamma photons from positron emitting radionuclides in the heart interact with other thoracic structures causing deviation from the original course of emission. This leads to a reduction in counts detected by the scanner. The attenuation of gamma photons from a uniformly dense object depends on the thickness of the object, irrespective of the depth of the source. To allow for this attenuation, the correction factors are measured directly, given the variation of density of thoracic and thoracic wall structures. A blank scan across the field of view is acquired with the exposure of an external ring source of germanium-68 (half-life = 287 days). Positrons from this external ring annihiliate in the air of the field of view and are

detected. This need only be done every week of scanner use. The next step of attenuation correction is the transmission scan which again uses the external ring source but with the patient positioned in the scan at the beginning of each study (Figure 3.2). Using the ratio of the counts from each coincident detector pair when the patient's body is present (transmission scan) and absent (blank scan), the correction factor for each detector is calculated for the degree of attenuation by different intrathoracic structures. This accurate process of attenuation correction along with improved spatial resolution is what sets PET above other radio-imaging techniques such as single photon emission computed tomography (SPECT).

3.4.4. Cross-calibration of the PET Camera

The PET camera measures counts of activity which by using the methodology described above leads to the accurate measurement of the true coincidence count rate. In order to allow quantitation, it is important that the camera is calibrated to permit calculation of radioactive concentration from count rate. This is done using a cylindrical phantom of a positron-emitting radionuclide at a known concentration, a sample of which has also been measured in a sodium iodide crystal well counter. The activity of the phantom from the camera is measured in counts·sec^{-1}·voxel^{-1} (the volume element equivalent of pixel in the reconstructed image), whereas the well counter measures radioactivity in μCi·ml^{-1}. This known relationship permits conversion from the count rate to absolute radioactive concentration.

3.4.5. Tracer Kinetic Modelling

The development of different radio-labelled tracers to exploit the advantages derived from the accuracy, repeatability, and quantitation of PET imaging is as important as improvements in the technical abilities of the PET scanners themselves. The definition of a tracer is that of a measurable compound which can follow ("trace")

a chemical process without markedly disturbing the process itself (Huang & Phelps, 1986). This is facilitated by the use of very low concentrations of PET tracers and the ability of cameras to perform rapid dynamic imaging to follow the chemical process under study.

Positron-emitting radionuclides may be incorporated into tracers by rapid radiochemical procedures and administered by the intravenous route or by inhalation. These then follow the natural chemical and metabolic pathways of the individual. As the amount of tracer delivered is known, the myocardial concentration (tissue response) and arterial concentration (arterial input) are measured as a function of time with the camera. This arterial input measured in left atrium or left ventricle documents the supply of tracer to myocardium through the study.

A tracer kinetic model is used to describe the relationship between the tissue response to the arterial input in mathematical terms, from which a quantitative assessment of the biological process studied may be derived. Such models are based on previous experiments with the tracer in both in vitro and in vivo (animal) models.

3.5 Limitations of PET Scanning

With an ideal imaging technique, the value in each pixel of a PET image is equal to the concentration of a positron-emitting radioisotope at that point in the tissue under study. However, no technique is free from a degree of error although with particular interpretation of images allowing for known sources of error, it is possible to minimize this.

3.5.1. Resolution

The primary limitation in PET is spatial resolution. The term line spread function (LSF) is used to define the spatial accuracy of a PET camera and refers to a narrow

line source of positron emitter between a pair of coincidence detectors to give a count rate profile as a function of position. In cylindrical detectors, the LSF is approximately gaussian in shape with a full width at half maximum (FWHM) of 40-50% of the diameter of the detectors at the midpoint of the detectors. Thus, LSF measurements taken over the central third of the region between coincidence detectors are remarkably constant, and that region constitutes the most useful field of view for accurate measurements in PET. When two line sources of activity are exactly 1 FWHM apart, it is not possible to tell that there are two sources, and when they are 2 FWHMs apart the lines should be clearly separated, with the valley between going to the background level.

The limiting resolution due to the physics of the PET system is of the order of 2-3 mm. Poor resolution can affect quantitative measurements by i) causing difficulty in interpretation of the anatomy for identification of the structure of interest (a problem becoming relevant to cardiac PET with the increased interest in documenting transmural flow accurately), ii) failing to resolve two structures close to each other and incorrectly attributing activity from one structure to the other, iii) reducing the apparent isotope concentration in structures that are smaller than about twice the system resolution, iv) causing overestimation in size of structures smaller than twice the resolution distance of the PET system, and v) low sensitivity in the detection of small low contrast lesions.

Resolution may also be lost if the patient moves during any stage if the scan. This is reduced by placing the patient on a comfortable bed, using a head restraint to remove axial movement, and employing short scan times.

The partial volume effect which is another limitation of scanner resolution is discused below (Section 3.6.1).

3.5.2. Accidental and scatter coincidences/

3.5.2. Accidental and scatter coincidences

Accidental and scatter coincidences are the two primary sources of background noise in PET. Accidental coincidences occur due to the nature of detection of emitted gamma rays. To establish that annihilation photons are in coincidence, timing measurements need to be performed for thousands of combinations of detection pairs. The detector, a scintillation crystal such as bismuth germanate, absorbs a photon and generates an electronic pulse (timing signal) of precise width (through emission of light to a photomultiplier), which is summed with other pulses from other detectors possibly in coincidence. When in coincidence, the pulses sum to twice the amplitude, identified by a pulse height discriminator and stored as image data. Because of the decay time of bismuth germanate (300 nsec), there is an inherent delay between the annihilation event and the timing signal. The width of the timing signal is chosen so that most of the signal from true coincidences falls into the electronic coincidence time window. Inevitably, unrelated photons may also produce timing signals which overlap - accidental coincidences. These may be corrected for and subtracted from the total coincidences either by measuring the single event of the individual detectors or using parallel timing circuitry to delay one of the signals (Hoffman & Phelps, 1986) (Figure 3.4). Scatter coincidences occur through photons being deflected by the tissue of the subject, with very little energy lost as a consequence and detected similarly to true events by the detectors. It is dependent on the cross-sectional geometry of the patient, the distribution of activity, and the design of the PET system. Although scatter can be measured in detail using line sources placed in the field of view, an estimate can usually be made from the edges of the field of view and correction made accordingly (Hoffman & Phelps, 1986).

Design of the PET system is of utmost importance in minimising these artifacts. A larger system diameter increases the ratio of true to background events an allows the

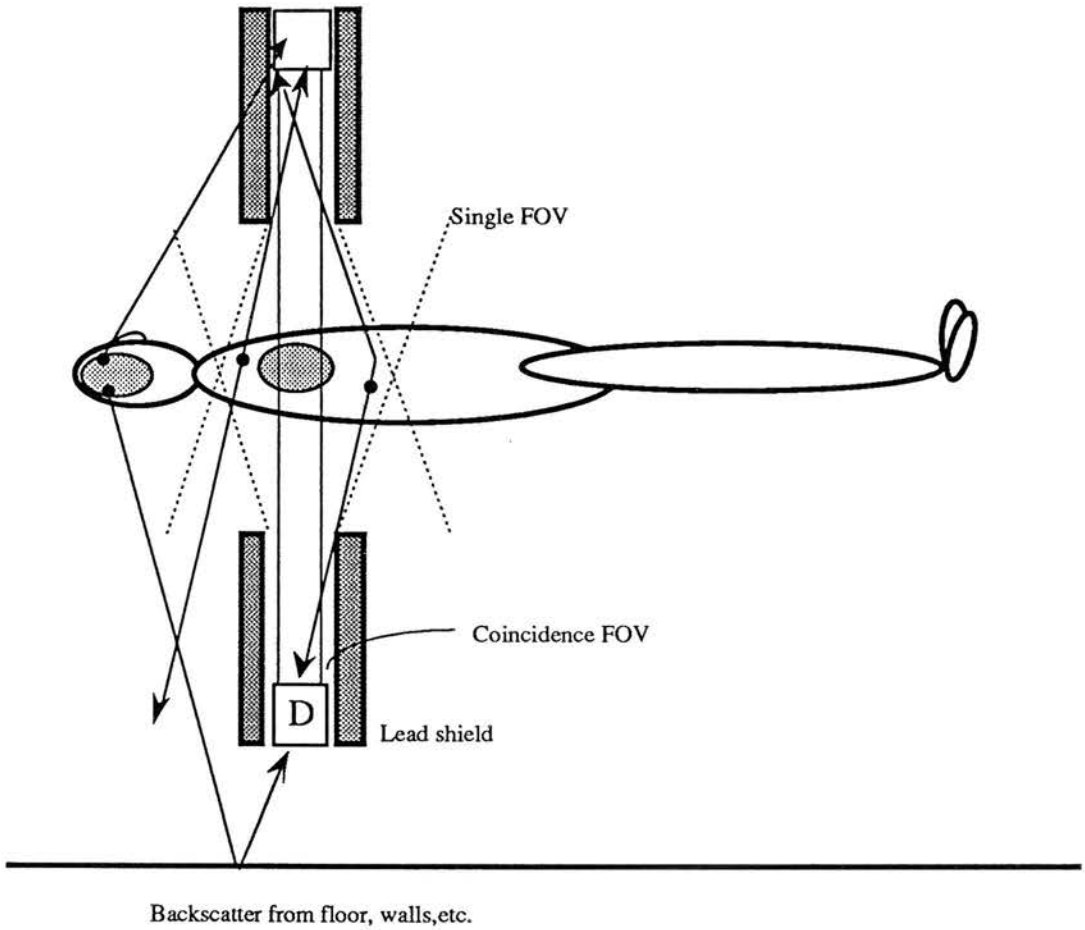


Figure 3.4. Sources of true, accidental, and scatter coincidences. True coincidences are those which are detected simultaneously in the region between the two detectors (D), the coincidence field of view (FOV). Accidental coincidences originate in areas of the subject which is in the direct field of view of one of the detectors (single FOV within either set of the dashed lines). Such coincidences can also arise from poor shielding, back scatter, and non-annihilation photons from the radioisotope. Scatter coincidences originate in a region that is viewed simultaneously by both detectors (between two sets of dashed lines) (Modified from Hoffman & Phelps, 1986)

use of longer lead side shielding. The sources of true, accidental and scatter coincidences are shown in Figure 3.4.

3.5.3. Dead-time losses

Dead-time losses can be estimated for a particular distribution of activity by taking a series of PET measurements of that distribution as a function of time until the total number of events per data acquisition is decaying with the true physical half-life of the isotope. Extrapolation to earlier times, with the physical half-life of the isotope, allows calculation of the true data rate. The difference between the true and measured rates is an estimate of data losses at higher count rates. Dead-time corrections in PET require simultaneous measurement of the single event rate of each detector and the coincidence rate.

3.6 Myocardial PET Scanning

The anatomical and physiological nature of the heart impose specific requirements on the acquisition and analysis of images generated by positron-emitting isotopes. Because of this, there are several considerations which need to be taken into account in order to improve image quality and tissue quantitation. It is important to ensure that the patient under study remains still during imaging so as to achieve optimum time-activity curves in each region of interest during each individual component of the study. In addition with multiple studies during the same scan, it is important that comparability is not lost between studies because of patient movement. To avoid this, a neon laser beam is used to demonstrate the patient's position in relation to the field of view, and temporary marks applied to maintain position from scan to scan.

The block detectors are positioned in a circumference around the long axis of the patient. However, the long axis of the heart is oblique to this such that images when

reconstructed are transaxial limiting complete evaluation of the left ventricle, particularly with respect to the inferior wall, cutting a tangential plane through this region. This problem is less apparent when restricting analysis to mid-ventricular sections with equally sized regions of interest from all walls of the ventricle. It is now possible to reslice the reconstructed PET images along the long axis of the heart to create short axis images from apex to base, without the need for tilting of the imaging gantry as was previously done in some centres.

Because the position of the heart in relation to the other thoracic structures is asymmetrical surrounded by tissue of different density, the degree and extent of photon attenuation by these tissues requires measurement before delivery of tracer, which has the advantage of conferring quantitation on the technique. This has lead to the initial transmission scan generated by external irradiation which allows determination of the attenuation coefficients of the soft tissues and correction for photon attenuation, as well as defining the thoracic anatomy.

Cardiac and respiratory wall motion are potential causes of image degradation with problems of motion artifacts causing apparent heterogeneity in myocardial tissue tracer uptake. PET images may be taken gated to the ECG, although breath-holding is not practical for most cardiac patients over the periods of time required for data acquisition. Another advantage of ECG-gating is that apparent changes in regional tracer concentrations caused by regional wall motion abnormalities and due to the partial volume effect (Section 3.6.1) may be minimised.

In addition to the potential problems caused by the physical nature of scanning, there are other considerations in relation to the analysis of images when determining true tissue concentration of tracer such as the partial volume effect, bidirectional cross contamination of activity between myocardium and blood, so-called "spillover" activity, and blood activity in the vascular space of myocardium.

3.6.1. Partial Volume Effect

Tissue concentrations of tracer are underestimated if the object size viz. left ventricular wall thickness, is less than twice the spatial resolution of the imaging camera (2 x FWHM), and the image is only partially occupying the sensitive volume of the detectors viewing that dimension, as after reconstruction, the concentration seen in the image is proportional to the concentration in the object. This leads to an underestimation of the isotope concentration in the object. Previously the resolution was of the order of 15-20 mm, causing underestimations of up to 50% of tissue tracer concentration. With improved spatial resolution, currently at 6.6 mm, this error has declined. In order to correct for the partial volume effect, corrections can be derived from scanning of different-sized phantoms containing a positron-emitting radioisotope allowing measurement of count recovery (the recovery coefficient) against object size.

In the method employed in this thesis, partial volume may be corrected for by manipulating the transmission and $C^{15}O$ blood pool images (Figure 3.5). This involves pixel-by pixel subtraction of a blood density image image from the transmission image after normalisation of the latter for tissue density (Iida et al, 1991). This provides a quantitative image of extravascular tissue density (Figure 3.5). Using phantom studies, this method has been validated over a range of wall thickness of 3-27 mm (Spinks et al, 1991). The value of extravascular tissue density measurement is dependent only on cardiac wall motion and the physical dimensions of the heart wall and is termed the anatomical tissue fraction (ATF; Section 3.9.1).

3.6.2. Spillover and Arterial Activity

The resolution of the scanner determines the amount of spillover of activity from the ventricular chamber ie. blood pool to the myocardium and vice versa. With the improvement in resolution with the newer generation of scanners, spillover has

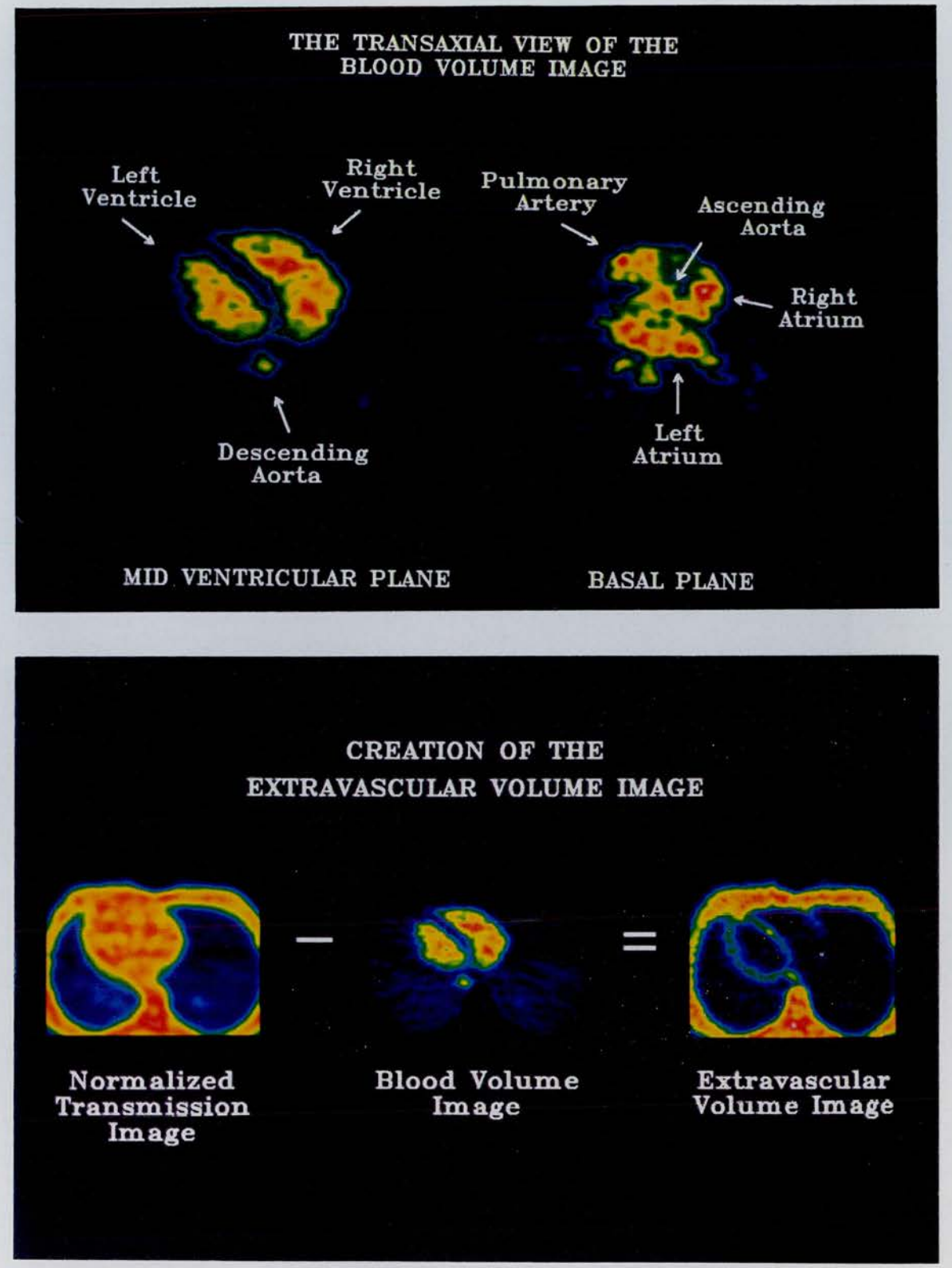


Figure 3.5. Upper panel) The blood volume or "blood pool" image is generated from the $C^{15}O$ inhalation scan and is reconstructed over 15 transaxial planes. A left atrial region of interest may be drawn over several planes to allow measurement of the arterial input function. **Lower panel)** The extravascular volume (or density) image is created by subtracting the blood volume image from the transmission scan, which is generated by external irradiation of the thorax by a 68-germanium ring source.

become less of a problem, and may be corrected for by modelling appropriately (Appendix 1).

In man, the coronary vascular space occupies an average of 10% of myocardial volume. High activity in this compartment contaminates the data from tissue activity, particularly early in tracer uptake. However, analysis of data over the whole of the dynamic image reduces this as a potential source of error (Iida et al, 1988).

3.7 Application of PET to the Measurement of Myocardial Blood Flow

Positron emission tomography offers the unique opportunity to evaluate myocardial perfusion in vivo with absolute quantitation, unavailable with other radio-imaging techniques (Bergmann et al. 1985). With the transverse field of view obtained with the current PET scanner, it is possible to measure myocardial perfusion in the whole heart and also measure perfusion in different regions of the left ventricular wall simultaneously.

Early work investigating the effect of coronary artery stenoses on MBF distribution used rubidium-82 (Selwyn et al, 1982), and nitrogen-13-labelled ammonia ($^{13}NH_3$) (Krivokapich et al, 1989), to demonstrate exercise-induced relative defects in myocardial distribution. However, the accumulation in ischaemic areas may reflect altered metabolism rather perfusion alone (Bergmann et al, 1985). This has led to the use of oxygen-15-labelled water ($H_2{}^{15}O$) which is a pure flow marker free from distortion attributable to the effects of ischaemia or metabolism (Bergmann et al, 1984; Iida et al, 1988). MBF measurement by $H_2{}^{15}O$ can be performed during vasodilator stimuli in order to assess the coronary vasomotor response. Quantitation of MBF during vasodilator or vasoconstrictor stimuli will allow comparison of the vasomotor response in the myocardial region under study with that of normal regions. Thus, by

comparing flow between these two regions, the behaviour of flow in the abnormal area may be referred to that of the normal regions (Walsh et al, 1990).

3.7.1. Myocardial Blood Flow Measured by Positron Emitters

There are three principles of an imaging method for accurately detecting regional changes in the coronary vasodilator reserve: i) cross-sectional PET to obtain depth-independent resolution of images, ii) a myocardial perfusion agent extracted by myocardium in proportion to flow at at levels up to 5 times basal values, and iii) a potent, consistent vasodilator stimulus to increase coronary flow sufficient to allow discrimination of myocardial flow between different regions (Gould et al, 1991). As PET perfusion imaging can discriminate a difference of only 15% in maximal flow between a diseased and control region, this makes it by far the most sensitive and specific radio-imaging technique for demonstrating the physiological consequences of a coronary stenosis.

Flow tracers may be extractable particulate, such as labelled albumin microspheres which need to be delivered directly to the left heart, or extractable diffusible, which are transienty trapped in or cleared from myocardium proportional to flow. Although many positron-emitting tracers have been developed to allow measurement of myocardial blood flow, many are impractical because of the need for intracoronary delivery to achieve sufficient myocardium to background ratios to allow external PET imaging. The tracers which have been developed for clinical use are ^{13}N-ammonia (half-life = 9.8 minutes), 82-rubidium (^{82}Rb; half-life = 1.3 minutes) and ^{15}O-water (half-life = 2.1 minutes).

3.7.1.1. ^{13}N-ammonia

^{13}N-ammonia given as an intravenous injection is cleared rapidly from from blood, with high myocardial retention giving high quality images. Although initial

extraction approaches 100%, there is some back diffusion leading to an extraction fraction of 82%. At higher flows, this declines further leading to a non-linear tissue response curve (the linear relationship only holds to myocardial blood flows of up to 2.5 $ml \cdot min^{-1} \cdot g^{-1}$). ^{13}N-ammonia diffuses across the cell membrane in the NH_4^+ form equilibrating to NH_3 which is trapped by the enzyme glutamine synthetase, which converts glutamic acid to glutamine. Because of the dependence on an enzyme involved in tissue metabolism, it has been suggested that tracer fixation may be affected by the underlying metabolic state of the myocardium (Bergmann et al, 1980). Under most circumstances such as changes in haemodynamics and contractility, the relationship between myocardial ^{13}N-ammonia net extraction and regional myocardial blood flow is not significantly affected. However, in chronic myocardial ischaemia, it may not be possible to make such assumptions.

^{13}N-ammonia clears rapidly from blood and distributes in proportion to regional myocardial blood flow in myocardium where it becomes trapped for several hours. Tissue concentrations of ^{13}N are stable within two minutes of injection. However, tissue concentration of ^{13}N can increase with time because of recirculation of labelled amino acids leading to error. Furthermore, arterial blood samples are required within two minutes of injection to allow well counting of ^{13}N activity.

3.7.1.2. 82-rubidium (^{82}Rb)

As rubidium has an extraction fraction of over 50%, it distributes in myocardium in proportion to blood flow and has been used for qualitative evaluation of myocardial perfusion. As ^{82}Rb is generated from a ^{82}Sr-^{82}Rb generator, it can be used for PET studies in centres without an on-site cyclotron. With a half-life of 78 seconds, it may be used for serial studies. To develop a method for quantitation of myocardial blood flow, two approaches have been used. The equilibrium technique relies on an image being taken during constant ^{82}Rb infusion, followed by another image after the

infusion is stopped. These two images allow calculation of the arterial and myocardial ^{82}Rb concentrations and thus the extraction fraction. This method relates the net regional myocardial uptake of ^{82}Rb for a given flow to the amount given. The bolus injection technique employs a rapid injection over 10-30 seconds along with sequential PET image acquisition. Both methods assume a constant extraction fraction, but extraction decreases non-linearly with higher flows leading to problems with accurate quantitation at higher blood flows. This has led to the development of a tracer kinetic model.

3.7.1.3. ^{15}O-water

^{15}O-water is freely diffusible with an extraction fraction approaching 100% which is unaffected by tissue metabolism. With a half-life of only 123 seconds, serial studies may be performed. Problems with this tracer originally occurred because of the high ^{15}O content of the blood pool and the myocardial vascular space. However, by recording blood pool images during ^{15}O-labelled carbon monoxide, it is possible to subtract blood pool activity from tissue activity (Figure 3.6). ^{15}O-water has been given as an intravenous bolus along with fast PET scanning, although this required intra-arterial sampling to determine the arterial input function (Iida et al, 1988). By giving a ^{15}O-labelled carbon dioxide ($C^{15}O_2$) inhalation which is instantly converted to ^{15}O-water in the lung capillaries, the delivery to the myocardium is achieved without the need for rapid scanning and high levels of activity. The advantages of this method of delivery are that $C^{15}O_2$ is easy to produce and may be delivered to the PET laboratory without the need for direct handling by the radiochemist, and may be controlled by the scanner operator. The slow inhalation, by providing a slow input function, reduces the artefact seen with bolus injections (Figure 3.7).

The main disadvantage is the high level of activity in the cardiac chambers which interferes with the myocardial signal, because of the limited resolution of the scanners.

To correct for this, the Va, representing the arterial blood volume and the spillover fraction, has been introduced into the model. Myocardial blood flow is calculated by fitting the arterial input and tissue time-activity curves to a single tissue compartment tracer kinetic model, which includes corrections for the underestimation of tissue activity due to the partial volume effect and the spillover of activity from the left ventricular chamber into the myocardial wall. The model estimates myocardial blood flow, the fraction of exchangeable tissue, and the Va with the region of interest measured. The fraction of exchangeable tissue is the fraction of the region of interest that consists of tissue capable of exchanging water within the time period of the study. This tissue fraction accounts for different wall thicknesses and thus corrects for myocardial tracer underestimation because of the partial volume effect, obviating the need for an independent measurement of wall thickness. Another minor disadvantage is that image quality is poorer than that seen with $^{13}NH_3$ or ^{82}Rb because of the kinetic characteristics of the tracer and the rapid dynamic scanning involved (Figure 3.8). However, the ability to quantify myocardial blood flow, with a correlation coefficient of 0.91 against microspheres, makes up for this minor disadvantage. The non-invasive measurement of the arterial input function is also affected the partial volume and spillover effects, although these effects may be minimised by using the left atrial chamber which has a recovery of over 90%. A mathematical description of the application of ^{15}O-water delivery to the measurement of myocardial blood flow is given in Appendix 1.

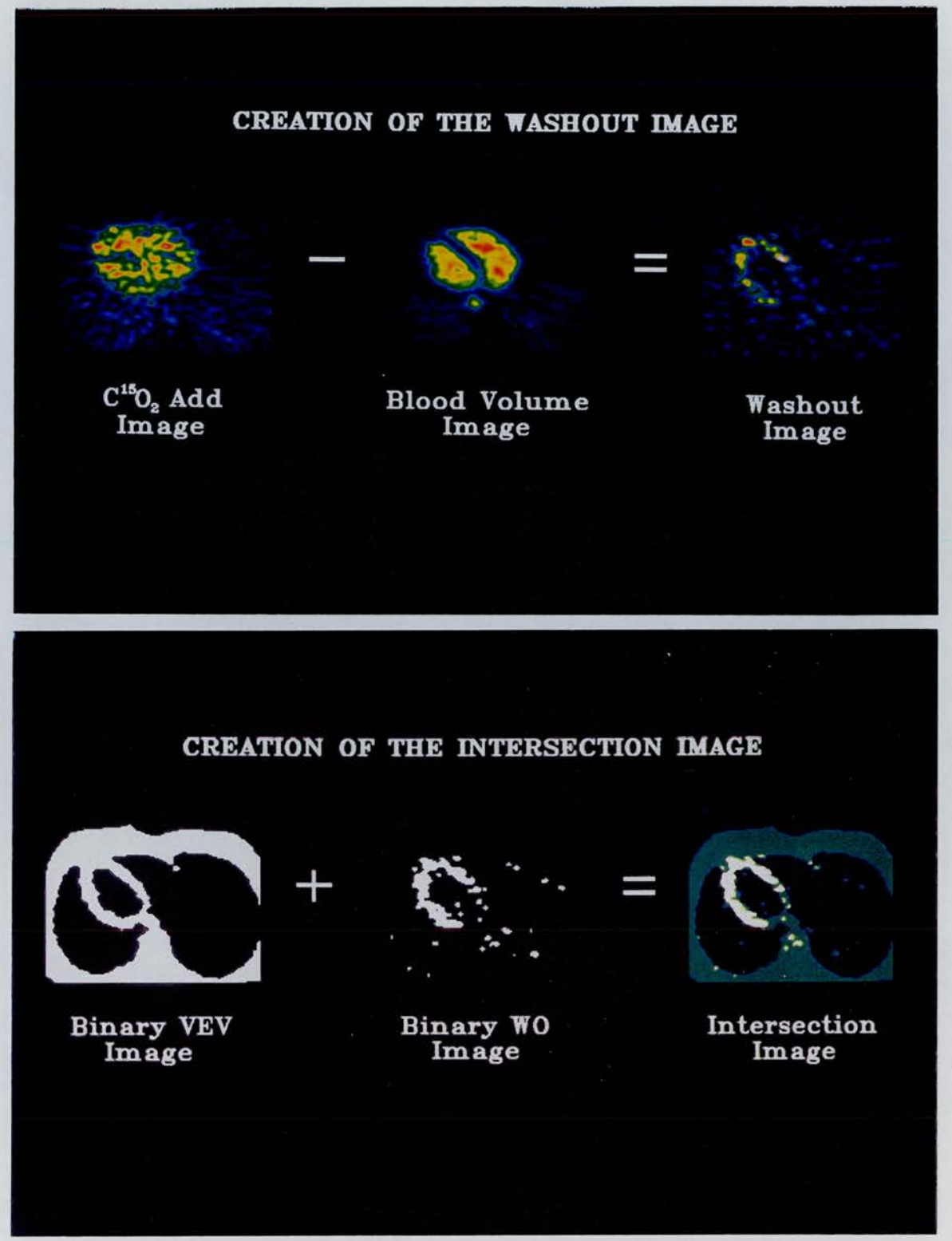


Figure 3.6. Upper panel) The dynamic $C^{15}O_2$ is acquired over a seven minute period consisting of i) a 30 second background scan, ii) inhalation of $C^{15}O_2$ over 210 seconds, and iii) a period of "washout" of the gas from myocardium over a further 180 seconds. The washout image is created from the last 4 time frames of this washout period, with subtraction of the blood volume image from the dynamic scan, to give an image of ^{15}O-water perfused tissue. **Lower panel)** To facilitate drawing of the regions of interest, the washout images eg. at basal and after vasodilator stress may be superimposed upon the scan of extravascular volume (vev) to produce an intersection image.

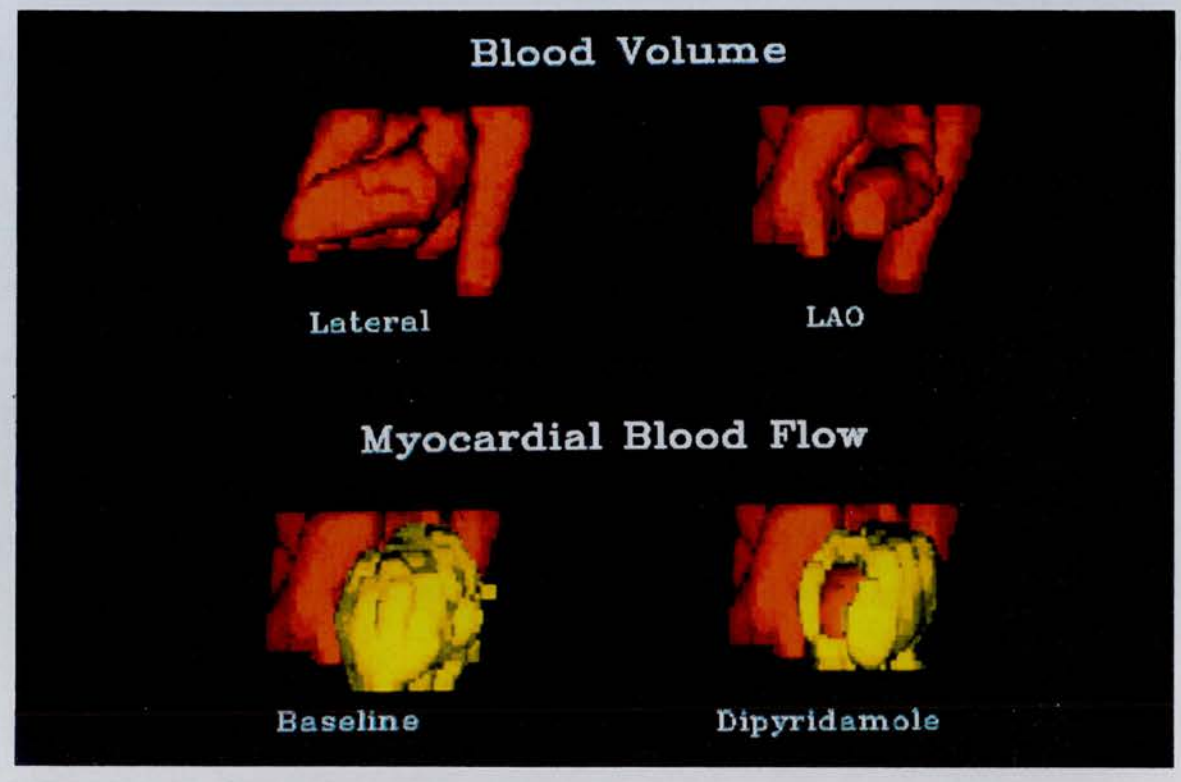
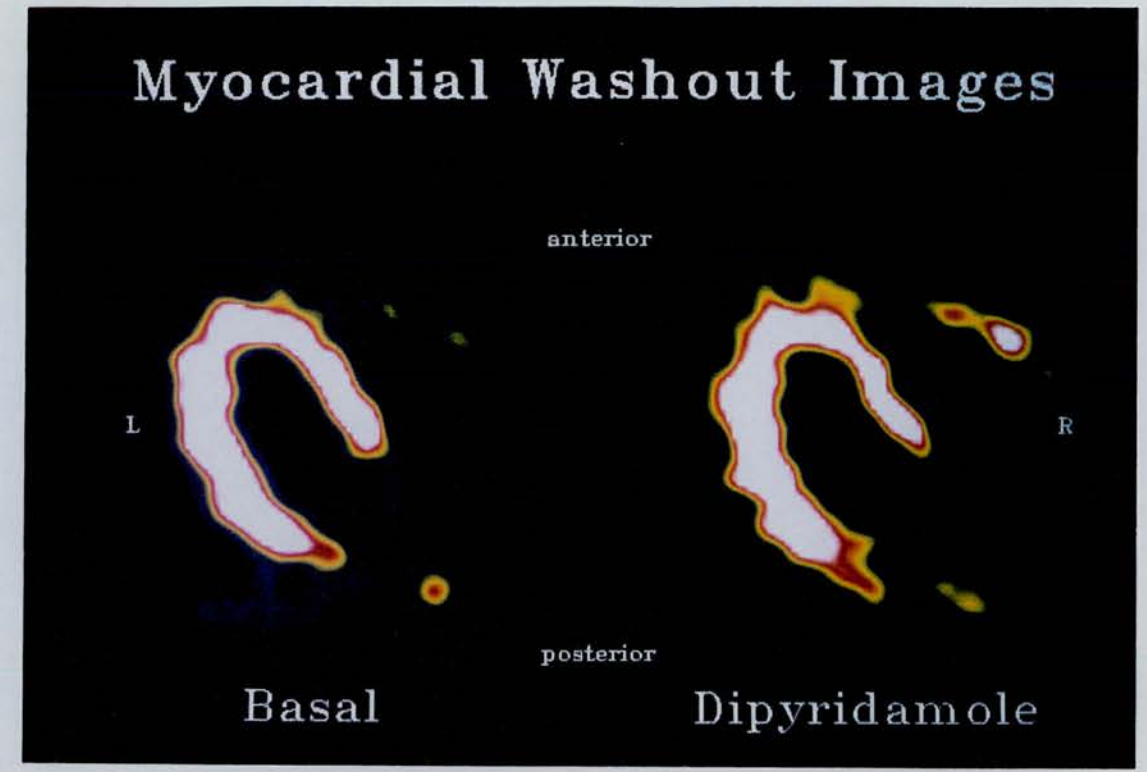


Figure 3.7. Three-dimensional images of blood volume (red image) in the lateral and left anterior oblique view (LAO) and myocardial blood flow or more correctly, washout images (yellow image) superimposed in the LAO view. The blood flow images are shown at basal and after the vasodilator stress of dipyridamole in a patient with a left anterior descending artery stenosis. In the latter image, the activity from the dynamic $C^{15}O_2$ scan is corrected to the flow in a remote region of myocardium. In the anterior region, myocardial blood flow fails to achieve this threshold and a perfusion defect is evident.

3.8 Application of PET to the Measurement of Myocardial Metabolism

PET has been used to study different metabolic processes in myocardium such as amino acid utilisation and protein turnover, but the main focus of attention has been the study of energy metabolism. PET also allows detection of the metabolic alterations caused by ischaemia and the definition of its three-dimensional myocardial distribution.

The glucose analogue 2-deoxyglucose, in which a hydroxyl group is substituted by a hydrogen atom on the second carbon atom, shares with glucose the same trans-sarcolemmal carrier and is a good substrate for the enzyme hexokinase with production of deoxyglucose-6-phosphate. This is not further metabolised and accumulates in the cell in proportion to exogenous glucose utilisation and hexokinase activity (Phelps et al, 1978). Although deoxyglucose uptake parallels glucose utilisation, it gives no further information regarding the disposal of glucose intracellularly. Nonetheless, global and regional changes in glucose utilisation under controlled conditions may be studied using deoxyglucose, labelled with the positron emitter, fluorine-18 (^{18}F-2-fluoro-2-deoxyglucose, FDG) (MacGregor et al 1977; Phelps et al, 1978) (Figure 3.8). Until recently, PET-derived glucose uptake measurements have been qualitative because of the difficulty in estimating a value known as the lumped constant. This factor relates the kinetic behaviour of FDG to glucose in the terms of their relative affinity for the trans-sarcolemmal carrier and hexokinase, and may vary under different physiological conditions. However, it is possible to quantify myocardial glucose uptake using a graphical method based on the measurement of fluorine-18 in the blood pool and myocardial regions of interest (Hicks et al, 1991). Of interest, it has been demonstrated that, even with the hyperinsulinaemic-euglycaemic clamp to control glucose and insulin levels, there is a decrease in glucose utilisation by the septum compared to the other regions, possibly



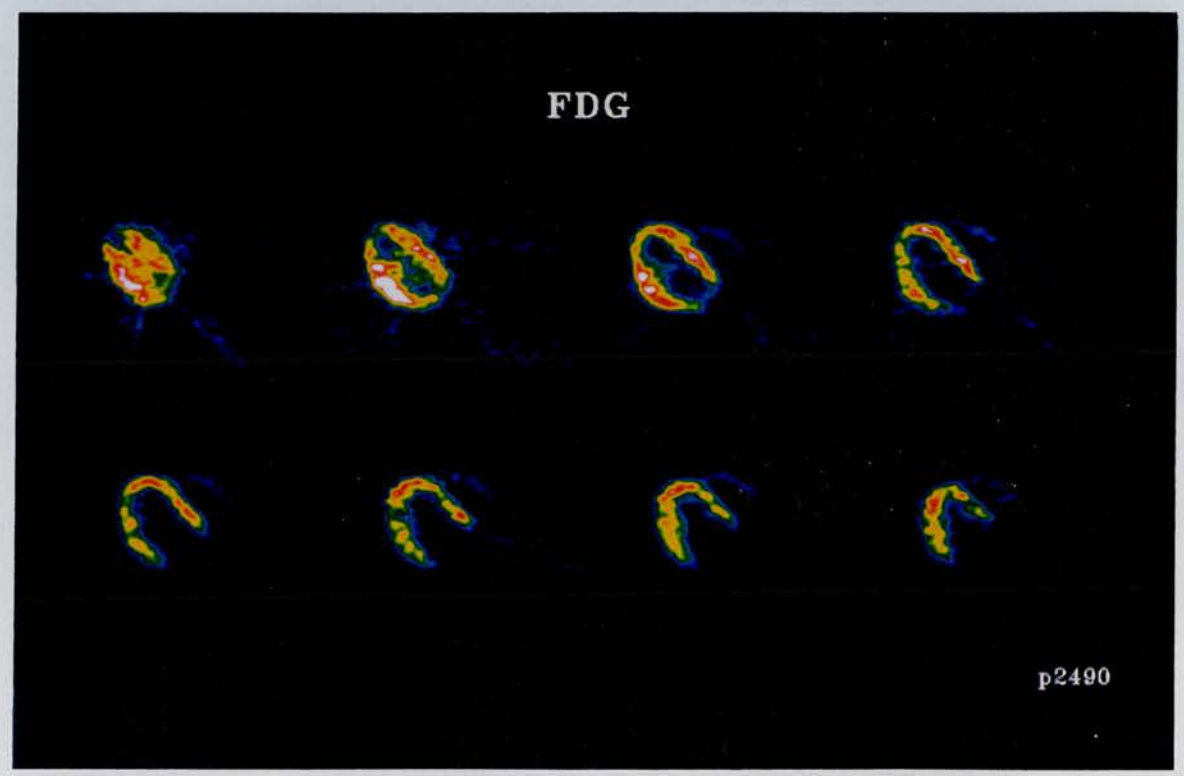


Figure 3.8. Upper panel) Myocardial washout images derived from $C^{15}O_2$ inhalation at basal and during dipyridamole stress at a mid-ventricular plane. **Lower panel)** FDG images over 8 planes from apex (upper left image) to base (lower right image) of the heart in a normal subject.

due to preferential free fatty acid utilisation (Hicks et al, 1991).

FDG imaging has been applied in two main areas; i) the demonstration of regional myocardial ischaemia in fasted individuals, and ii) the demonstration of reversibly injured, or viable myocardium in patients with previous myocardial infarction or reduced left ventricular function.

3.8.1. FDG Imaging and Myocardial Ischaemia

An epicardial coronary artery stenosis reduces the increase in coronary blood flow available during stress when an increase in myocardial oxygen demand occurs. Most of our knowledge on myocardial metabolism comes from work on isolated perfused animal hearts in normoxic and anoxic conditions. A description of normal cardiac metabolism with particular reference to myocardial carbohydrate metabolism is given in Appendix 2. Ischaemia is the commonest situation leading to reduced myocardial oxygen delivery in man, and many different factors determine the duration, severity and extent of ischaemia (Maseri, 1983). FDG is selectively taken up by myocardial cells which have been rendered ischaemic, even for a few minutes (Camici et al, 1986). This approach lends itself particularly well to the study of ischaemia caused by provocation tests that do not increase myocardial work, as this by itself could stimulate glucose uptake in non-ischaemic myocardial cells.

During myocardial ischaemia, major changes occur in intracellular substrate metabolism: there is an increased glycogen breakdown and exogenous glucose uptake which fuel glycolysis (Camici et al, 1989a; Opie et al, 1973) (Figure 3.9). Accumulation of reduced coenzymes ($NADH_2$) stimulate the conversion of pyruvate to lactate by lactate dehydrogenase. With mild ischaemia, the transport of glucose into the cell is accelerated, as is glycolysis. However, the distal products of glycolysis, lactate and reduced coenzymes, will finally inhibit the enzyme glyceraldehyde 3-phosphate dehydrogenase lower down the Embden-Meyerhof pathway (Rovetto et al, 1975).

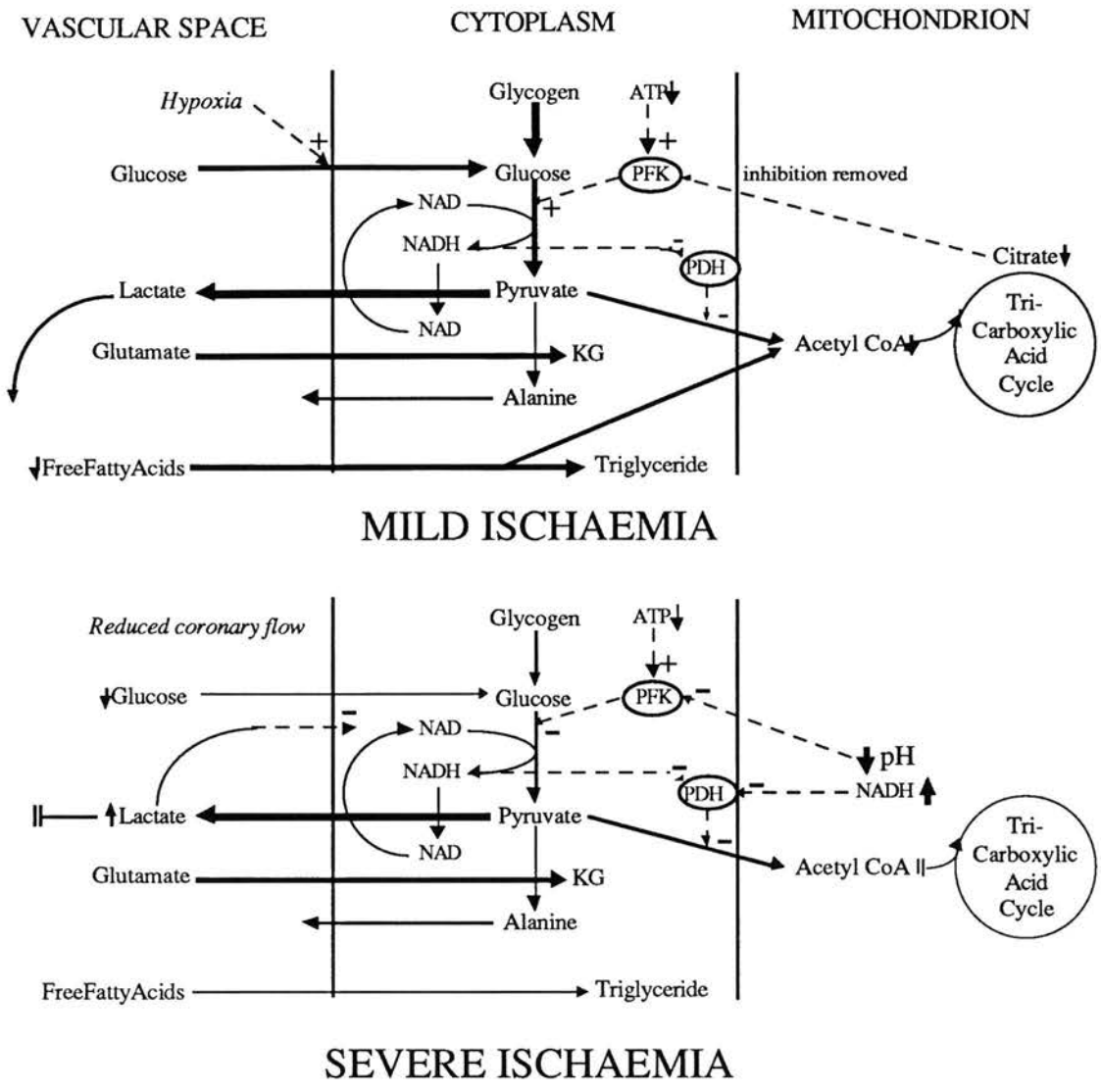


Figure 3.9. Upper panel) During stress-induced myocardial ischaemia, there is a reduced oxidation of free fatty acid with an increased storage of triglyceride. Glucose utilisation is increased (both from exogenous glucose and from glycogen), but the extra pyruvate formed cannot be oxidised. Hypoxia stimulates glucose uptake and phosphofructokinase (PFK) activity is accelerated due to a decrease in adenosine triphosphate and creatine phosphate and a decrease of citrate. Pyruvate dehydrogenase is inhibited by increased amounts of NADH (reduced coenzyme nicotinamide dinucleotide). There is an increased production of alanine with a greater myocardial glutamate consumption. In addition, due to the accumulation of NADH, there is activation of the enzyme lactate dehydrogenase, with conversion of pyruvate to lactate that is then released; at the same time 1 mole of NADH is reoxidised to NAD. **Lower panel)** With severe myocardial ischaemia, glycolysis is inhibited. This because of a reduced glucose delivery, inhibition of phosphofructokinase by a low intracellular pH and inhibition of glyceraldehyde-3-phosphodehydrogenase by lactate and NADH. (Modified from Camici et al, 1989 a,b).

Although there is an increase in glucose uptake, carbohydrate oxidation is negligible (Figure 3.9). With the return of myocardial oxygen delivery, lactate is oxidised back to pyruvate with restoration of glycogen to pre-ischaemic values. The time required to return to the pre-ischaemic metabolic pattern is prolonged with persisting high glucose uptake, probably for glycogen re-synthesis, more so in post-ischaemic than normal myocardium (Camici et al, 1989b). This persistence of high glucose uptake in post-ischaemic myocardium is the underlying principle which allows the identification of transiently ischaemic myocardium using FDG with PET.

With severe ischaemia, the rate of exogenous glucose delivery is also reduced (Figure 3.9). Under these circumstances, the ischaemic myocardium will rely almost exclusively on intracellular glycogen stores. However, the insufficient "washout" of accumulated hydrogen ions and the ensuing intracellular acidosis will inhibit phosphofructokinase, and thus, glycolysis much earlier than with mild ischaemia. Thus, the uptake and intracellular utilisation of glucose is modulated by the presence and severity of ischaemia.

3.8.1.1. Chronic Stable Angina

At rest in the fasting state, myocardial FDG uptake is comparably low in normal controls and in patients with angiographically proven coronary artery disease and stable exertional angina. After supine exercise (sufficient to induce chest pain and significant ST segment depression), a marked increase in FDG uptake may be seen in both normals and patients (Camici et al, 1986).

In patients, FDG uptake in non-ischaemic regions (regions with a normal increment of coronary flow on exercise) was similar to that in control subjects. However, FDG uptake was significantly higher in regions with impaired flow during exercise (defined as perfusion defects at rest). This disparity in FDG uptake was detected well into the post-exercise phase, after defects of the flow marker ^{82}Rb and

the ECG had returned to baseline. This increased uptake is thought to be due to prolonged recovery of metabolic function with a continued preference for glucose in "post-ischaemic" myocardium, and may reflect increased glycogen synthesis to replenish glycogen stores that were depleted during ischaemia and increased cardiac work (Camici et al, 1986). However, the precise time course of this phenomenon and its possible relation to the severity of the ischaemic episode is still to be elucidated.

Recovery of full contractile function after revascularisation may also be delayed until tissue glucose metabolism returns to normal. In another study of patients with stable coronary artery disease using $^{13}NH_3$ and FDG, it was observed that segmental wall motion abnormalities did not improve 48 hours after coronary angioplasty despite improvement in myocardial perfusion (Nienaber et al, 1991). However, wall motion scores had improved at two months follow-up associated with improvement in blood flow-FDG mismatch, implying that normalisation of the altered metabolic state is a prerequisite for contractile recovery. This may be evidence for slow normalisation of a chronic metabolic adaptation to a persistent reduction in blood flow (Fedele et al, 1988).

3.8.1.2. Unstable Angina

PET scanning with FDG has also been done in patients with severe coronary artery disease and unstable angina characterized by frequent spontaneous episodes of ST segment depression at rest. Unlike patients with stable angina pectoris with an FDG uptake at rest similar to control subjects, patients with unstable angina may have regional and even global increases in FDG uptake at rest in the absence of perfusion abnormalities or transient myocardial ischaemia (Araujo et al, 1988). As a control, glucose utilisation was similar in chest wall skeletal muscle in all groups studied. This increase in FDG uptake, of up to four-fold, occurred throughout the myocardium compared to patients with stable angina or control subjects, in the absence of

ischaemia, and despite similar disease severity at angiography. No relationship was seen between FDG uptake and recent episodes of chest pain or ST segment depression. This has led to the concept of a metabolic adaptation to chronic ischaemia (with preferential glucose utilisation) which is sustained even in the absence of demonstrable myocardial ischaemia (Fedele et al, 1988). It is of interest that an increase in glucose utilisation may occur in areas not subtended by a stenotic artery. This may imply that the metabolic derangement in areas supplied by diseased vessels imposes a metabolic stress on a normal area such that there is also a move towards an increased glucose flux, possibly because of a necessary increase in cardiac work, so-called regional intra-ventricular loading. Alternatively, this may be evidence for altered metabolism in territories subtended by angiographically normal arteries (Chapter 7).

3.8.1.3. Myocardial Infarction

Metabolic imaging with positron emission tomography has been of value in characterising the functional insult of myocardial infarction and the determinants of recovery. In animal studies using ^{15}O-water and ^{11}C-palmitate, after recanalisation following coronary occlusion, myocardial blood flow is reduced after initial high reflow. Although oxidative metabolism (^{11}C-palmitate) parallels this early rise and fall, the subsequent recovery of fatty acid accumulation is much more variable, but ultimately predictive of viability (Knabb et al, 1987). Despite often rapid normalisation of myocardial oxygen consumption (^{11}C-acetate) in infarcted tissue, there is a delay in recovery of myocardial blood flow. However, recovery of regional wall motion and oxidative metabolism occur in parallel (Buxton et al, 1992). In man, it has been confirmed that the preservation of oxidative metabolism is a necessary condition for recovery of regional function (Gropler et al, 1992: Vanoverschelde et al, 1992). These studies using direct measures of cellular oxidative metabolism (^{11}C-

acetate) substantiate the earlier studies done using FDG and PET as a clinical predictor of myocardial viability.

In contrast to the fasting state, there is regional uptake of FDG in the presence of cellular metabolism with a concomitant reduction where there is necrotic tissue consistent with infarction. The PET concept of viability is based on the demonstration of a maintained or increased glucose metabolism in areas of reduced perfusion ("mismatch"). On the other hand, a concordant reduction of myocardial blood flow and glucose uptake ("match") indicates the presence of scar without any viable tissue and predicts no recovery of function (Figure 3.10). In one study, Marshall et al demonstrated that within the first weeks after myocardial infarction, preserved glucose metabolism (relative to flow measured with $^{13}NH_3$) ie. mismatch, identified viable tissue (Marshall et al, 1983). In another study, early after myocardial infarction, although it was not possible to differentiate hypoperfused regions with preserved glucose metabolism from those without residual metabolic viability according to myocardial perfusion alone, segmental wall motion abnormalities, or left ventricular ejection fraction (Schwaiger et al, 1986), after 6 weeks, 50% mismatched segments improved their segmental wall motion compared to no change in the matched group. Thus, maintenance of metabolic activity in infarcted territory predicts a variable outcome consistent with the wide variation in residual perfusion, collateralisation and borderline ischaemia within 72 hours of infarction.

Consistent with the added prognostic value of an open artery after thrombolysis, PET scanning with FDG has demonstrated increased uptake in 76% of regions subtended by such an artery, compared to 29% of regions with an occluded artery, despite significant collateral flow in the majority of these (Schwaiger et al, 1987). Conversely, reduced or absent FDG uptake in an infarct territory is predictive for no improvement in ventricular function (Schwaiger et al, 1987; Pierard et al, 1990). These early studies have led to the use of PET as a means of predicting the success of

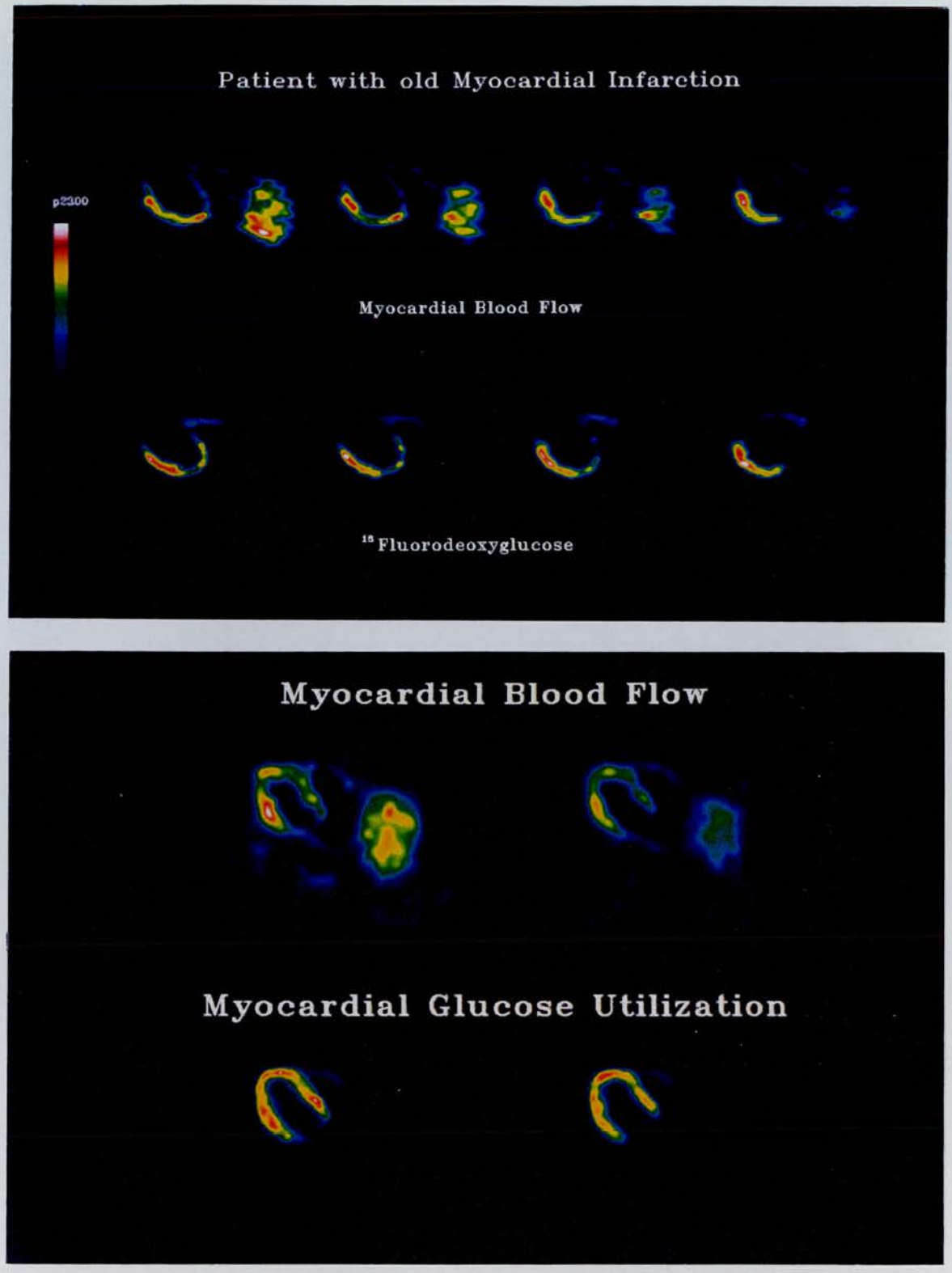


Figure 3.10. Upper panel) MBF washout and FDG images over four adjacent myocardial planes in a patient with a previous extensive anteroseptal myocardial infarction. MBF and FDG uptake is extremely low in the septal, anterior an apical regions, but equally preserved in the lateral and inferoposterior regions - "flow-metabolism match". **Lower panel)** MBF washout and FDG images in two myocardial planes in another patient with a previous anteroseptal myocardial infarction. MBF is low in the anterior and septal regions but FDG uptake is preserved in all myocardial regions "flow-metabolism mismatch".

revascularisation particularly in patients with poor left ventricular function or in those patients where conventional radio-imaging techniques may suggest irreversible myocardial injury (Section 3.9). The subject of PET imaging and the diagnosis of myocardial hibernation and thus myocardial viability has been covered extensively in recent reviews (Uren & Camici, 1992; Dilsizian &Bonow, 1993).

Thus, PET imaging can distinguish viable and non-viable myocardium on the basis of analysis of FDG and myocardial blood flow images after oral glucose loading. This concept of match and mismatch has been developed to predict the success of revascularisation and has become the main clinical indication for PET scanning (Section 3.9).

3.8.2. PET Imaging of Oxidative Metabolism

As described above, in addition to the imaging of glucose uptake using FDG, other tracers have been developed specifically to study myocardial oxidative metabolism, most notably ^{11}C-palmitate and ^{11}C-acetate. ^{11}C-palmitate has been used to trace the oxidation of free fatty acids which are the principle source of high energy phosphate generation in fasted individuals. However, kinetic modelling of this and related tracers has proven difficult making quantitation of oxidative metabolism impossible. ^{11}C-acetate has been developed to trace myocardial oxygen consumption as it enters the tricarboxylic acid cycle directly (Walsh et al, 1989; Armbrecht et al, 1989). Clearance of this tracer does correlate with oxygen consumption (Brown et al, 1987), but again absolute quantitation has also been difficult because of the early generation of $^{11}CO_2$ from aerobic metabolism of acetate. Because of this, there has been much interest in taking the study of oxidative metabolism one stage stage further with the development of $^{15}O_2$ itself as a direct means of quantifying regional myocardial oxygen consumption. The development of this latter tracer is outwith the scope of this thesis.

3.9 New Developments in the Measurement of Myocardial Viability

In the clinical environment, most PET scanning is done to demonstrate myocardial viability in patients with reduced left ventricular function prior to revascularisation. The use of the mismatch of blood flow to FDG uptake as an index of viability (Section 3.8.1.3) has been used to predict the success of revascularisation. Tillisch et al studied 17 post-infarction patients where preservation of glucose uptake predicted recovery of 85% of asynergic segments with flow-metabolism mismatch after coronary artery bypass grafting. Decreased glucose uptake (flow-metabolism match) implied irreversibility of hypokinesis in 92% of cases (Tillisch et al, 1986). This was confirmed by Tamaki et al in another study of 22 patients before and 5-7 weeks after surgery with a positive predictive accuracy of 78% of flow-metabolism mismatch (Tamaki et al, 1989). They reported a lower negative predictive accuracy of 78% than the 92% quoted by Tillisch et al (Tillisch et al, 1986). This difference probably reflected studying patients in the fasted state with a much lower uptake by normal myocardium increasing the sensitivity of FDG uptake, but in areas with not sufficient viability to improve with revascularisation. Recovery of myocardial segments after coronary angioplasty has also been predicted (Nienaber et al, 1991). In this study, the degree of flow-metabolism mismatch before angioplasty was directly related to late improvement in wall motion, thus allowing quantitative prediction of contractile recovery. Other studies have shown flow-metabolism mismatch in asynergic regions with fixed thallium-201 defects, indicating that conventional radio-imaging techniques tend to underestimate the extent of viable myocardium (Brunken et al, 1987). Furthermore, by quantifying flow and to an extent metabolism, and deriving a ratio of the two, viability may be defined as occurring in asynergic regions with metabolism:flow ratios of ≥ 1.2 (Bonow et al, 1991; Vanoverschelde et al, 1992).

Myocardial FDG uptake depends on dietary conditions (whether the patient is fed or fasting which determines substrate availability), the neurohormonal state, cardiac workload, and the presence of diabetes mellitus (Camici et al, 1989b; Pierard et al, 1990). Even in the normal heart under fasting conditions, there is variability in regional myocardial glucose uptake independent of oxidative metabolism or perfusion with reduction in FDG uptake in the interventricular septum (Gropler et al, 1990). Even so, in comparison with ^{11}C-acetate, this reduction in septal FDG uptake persists despite a similar metabolic demand to other regions studied (Hicks et al, 1991). Furthermore, it has been shown recently in an animal model of coronary occlusion and reperfusion that myocardial deoxyglucose deposition in infarct territories overestimates the extent of myocardial necrosis at histology (Sebree et al, 1991). In addition, it was shown that deoxyglucose uptake was increased in peri-infarct regions. Detection of myocardial viability using FDG is dependent on the demonstration of residual FDG uptake in the area at risk. However, the FDG uptake in infarct regions may occur due to residual deoxyglucose uptake in persisting ischaemic tissue or inflammatory cells (Sebree et al, 1991). In addition, the presence of FDG activity in an infarcted region may result from the limited spatial resolution of PET cameras. This may produce a spillover of activity from the peri-infarct regions, which have an enhanced FDG uptake, into the infarcted region on the reconstructed FDG image.

These observations may make assessment of myocardial viability with FDG less accurate, and are a strong argument for standardisation of study conditions by using the hyperinsulinaemic-euglycaemic clamp technique (de Fronzo et al, 1979), which improves the interpretation of FDG images when comparing control and diseased regions. However, despite such improvements, the search for other measurements of myocardial viability has continued.

3.9.1. The Perfusable Tissue Index

In order to measure viability without using FDG and consequent problems with interpretation, a new measure, the water perfusable tissue index (PTI) which is independent of tissue metabolism has been developed derived from the analysis of transmission, blood pool ($C^{15}O$) and dynamic ($H_2{}^{15}O$) scans (Yamamoto et al, 1992). The concept of tissue fraction was first introduced to correct for the underestimation of myocardial radiotracer concentration caused by cardiac wall motion and the small transmural myocardial thickness relative to the spatial resolution of PET scanners (the partial volume effect, Section 3.6.1) (Iida et al, 1988). Tissue fraction is the fractional volume of a given region of interest occupied by myocardium that is capable of rapidly exchanging water ie. the $H_2{}^{15}O$-perfusable tissue. It is dependent on the ability of the tissue to exchange $H_2{}^{15}O$ at a microvascular level as well as wall motion and partial volume effects. It may be referred to as perfusable tissue fraction (PTF). Using the anatomical tissue fraction (ATF) defined above it is possible to define the PTI as:

$$\text{PTF } (g(H_2{}^{15}O\text{-perfusable tissue})\cdot ml^{-1}\ ROI) \div \text{ATF } (g(\text{anatomical tissue})\cdot ml^{-1}\ ROI)$$

In normal myocardium, this value should be unity ie. all myocardium should be perfused by water. Using $H_2{}^{15}O$ as a flow tracer, this technique also allows absolute quantification of myocardial blood flow, in addition to a measure of myocardial viability (Figures 3.11 and 3.12).

In an initial study, patients were studied within 2-4 days and 4 months after successful thrombolysis for myocardial infarction who had asynergic myocardium (at echocardiography) and a regional reduction in myocardial blood flow (Yamamoto et al, 1992). In the acute phase, the PTI was significantly lower in regions in which there was no subsequent contractile recovery, although myocardial blood flow was reduced to a similar extent in both recovery and non-recovery segments. These data also

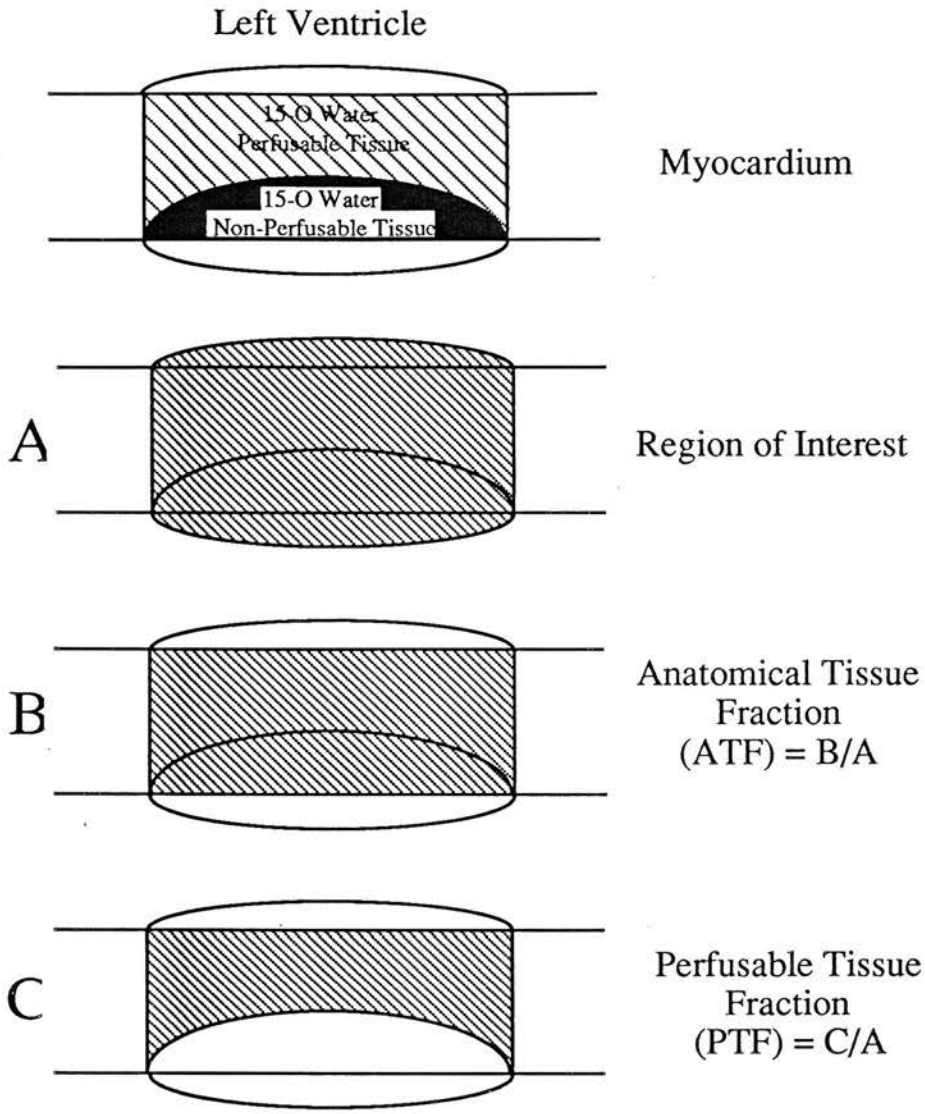


Figure 3.11. Diagrammatic representation of a myocardial region of interest (ROI) containing a mixture of ^{15}O-water perfusable and non-perfusable tissue. **Panel A)** Volume of the ROI. **Panel B)** Anatomical tissue fraction (ATF) for the ROI produced by subtraction of the converted $C^{15}O$ emission scan (blood pool) from the transmission data after normalisation of the latter to tissue density. ATF contains both perfusable and non-perfusable tissue components. **Panel C)** ^{15}O-water perfusable tissue fraction (PTF) for the ROI calculated from the dynamic $H_2{}^{15}O$ study. Note that the non-perfusable or necrotic region is excluded from this parameter. The ^{15}O-water perfusable tissue index (PTI) is calculated by dividing PTF (C/A) by ATF (B/A) thus independent of ROI size, and represents the fraction of total anatomical tissue that is perfusable by water.

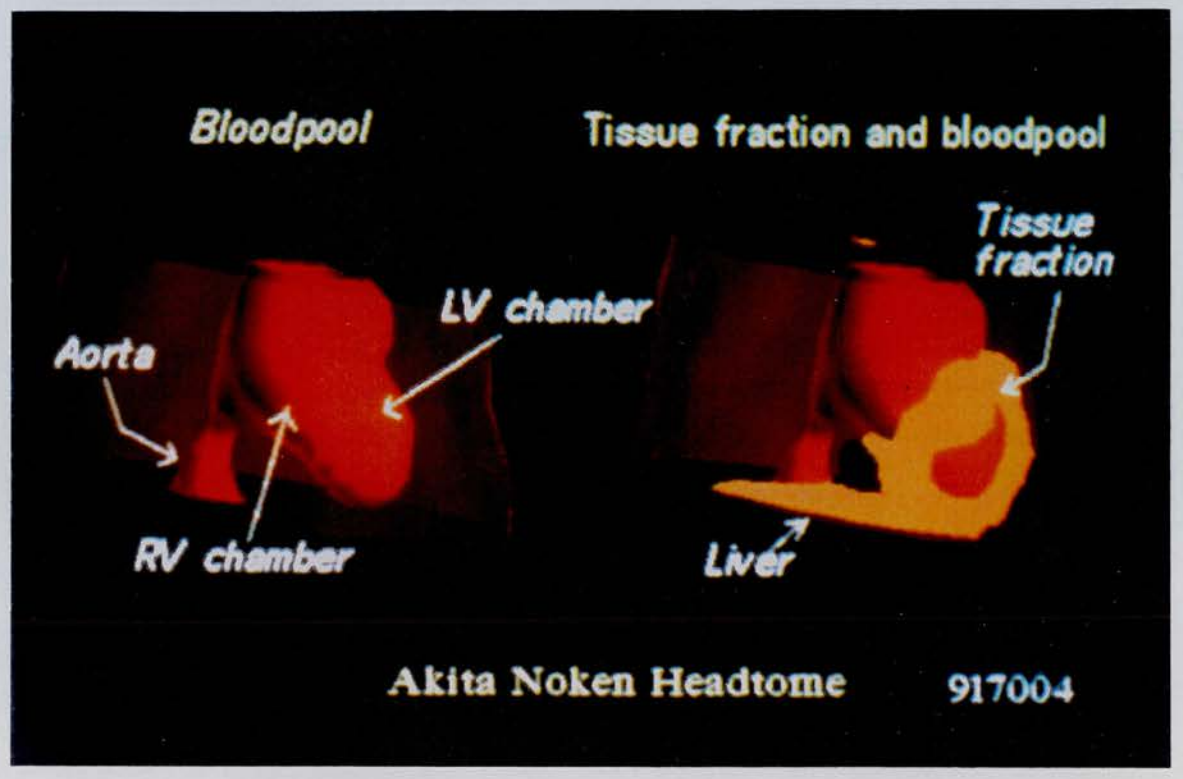


Figure 3.12. The PTI may also be demonstrated as a three-dimensional image corrected to a lower threshold of 70% water-perfused myocardium (yellow image). This is superimposed on the blood volume image (red image) in the right anterior oblique view. The subject has sustained a small anterior myocardial infarction with an apparent "window" of <70% water perfused tissue ie. non-viable myocardium.

indicated that within a given region, 70% of the tissue should be capable of rapidly exchanging water to enable a subsequent improvement in contractile function. In patients with previous myocardial infarction, PTI demonstrated good agreement with FDG scanning in predicting reversibility of injury in these patients. Subsequently, this sensitivity in discriminating reversibly from irreversibly injured myocardium has been confirmed in a group of 12 patients undergoing coronary bypass surgery and angioplasty (de Silva et al, 1992). In this study, contractile recovery only occurred in myocardial regions where PTI was greater than 0.7.

Given that there is a coincident "downregulation" of metabolic activity in hibernating myocardium, there is a concomitant reduction in tissue oxygen utilisation. It is possible to directly measure myocardial oxygen consumption non-invasively at PET using the tracer $^{15}O_2$ (Yamamoto et al, 1991). As described above, flow-metabolism mismatch can demonstrate viability in qualitative terms but with limited accuracy. By using oxygen utilisation as an absolute measure of regional tissue metabolism, independent of substrate availability and glycolytic rate, it may be possible to differentiate hibernating myocardium from non-viable tissue with greater accuracy. Combining measurement of regional oxygen consumption with the PTI derived from dynamic $H_2{}^{15}O$ imaging should allow better discrimination of chronically ischaemic tissue from infarction and the amount of viable tissue in an infarcted area. The combination of measurement of regional wall motion by gated PET scanning, myocardial blood flow and the perfusable tissue index with $H_2{}^{15}O$, and regional myocardial oxygen utilisation with $^{15}O_2$, will provide accurate assessment of myocardial viability, the determination of which is likely to remain the one major clinical indication for PET scanning in the future.

3.10 Summary

The basic principles of PET and the design of the tomograph used in our institution have been described. By incorporating positron-emitting isotopes into metabolic substrates, it is possible to measure regional myocardial blood flow, and myocardial oxidative metabolism, and by using radiolabelled ligands, the pharmacokinetics of myocardial receptor function. The advantage of quantitative assessment of myocardial perfusion allows the accurate study of the control of myocardial blood flow under basal conditions and, with the short half-lives of tracers, the serial study of myocardial blood flow after stress, and thus the assessment of coronary resistive vessel function. Combined with the demonstration of regional myocardial ischaemia with FDG, it is now possible to investigate the mechanisms underlying cardiac disease, with particular reference to atherosclerosis, and coronary artery disease.

CHAPTER 4. MODELS FOR THE STUDY OF RESISTIVE VESSEL DYSFUNCTION IN CORONARY ARTERY DISEASE

4.1 Introduction

Recent observations in patients have led to the hypothesis that the coronary vasodilator reserve and thus ischaemic threshold can be substantially altered by changes in vasomotor tone at the level of the resistive vessels rather than in large epicardial coronary arteries. There are several models where the role of dynamic epicardial coronary stenoses can be excluded. By studying different clinical models of coronary artery disease, it is possible to determine the role of resistive vessel dysfunction in causing abnormalities of coronary blood flow in these conditions. It is also possible to determine to what extent they account for other abnormal findings and their association with other markers of cardiac function. The determination of the contribution and mechanisms of resistive vessel dysfunction to the coronary artery disease syndromes, may enable new therapies to be developed which might ameliorate this abnormality and thereby reduce morbidity.

4.2 Proposed Models in Coronary Artery Disease

Several models have been selected for study in this thesis, in order to investigate coronary resistive vessel dysfunction in different manifestations of the process of coronary artery disease. These have been selected so that resistive vessel function and its correlate, the coronary vasodilator reserve, may be assessed in regions of myocardium subtended by diseased and angiographically normal coronary arteries

using positron emission tomography. In selected models, Doppler catheterisation has been used to investigate further the mechanism of abnormalities in coronary blood flow in epicardial vessels, and coronary sinus catheterisation performed to quantitate regional myocardial metabolism. The following five studies comprise the work of this thesis.

In Chapter 5, the first model studied is of patients in whom a flow-limiting coronary artery stenosis has been relieved by successful percutaneous transluminal coronary angioplasty. In a previous study following successful angioplasty, the coronary vasodilator reserve determined using intracoronary papaverine did not return to normal in 55% of patients immediately following the procedure (Wilson et al, 1988). In the absence of restenosis, the coronary blood flow reserve had returned to normal at a mean follow up of 7.5 months. The authors suggested that abnormal resistive vessel function may account for this finding, and that prolonged vasodilatation resulting from low perfusion pressure distal to the stenosis might cause a transient inability of the resistive vessels to autoregulate in response to a sudden restoration of normal perfusion pressure (Wilson et al, 1988). These findings are consistent with the observation that a positive exercise test may be demonstrated as early as three days after successful PTCA and remain positive for up to a month before becoming negative, in the absence of restenosis or coronary artery spasm using non-invasive provocation tests (El-Tamimi et al, 1991). In this study, our intention was to investigate the changes in coronary resistive vessel function which occur immediately after angioplasty and to determine the time course of any changes in resistive vessel function.

In Chapter 6, the mechanism underlying this alteration in resistive vessel function after angioplasty is investigated. Deficient production or release of endothelium-derived relaxing factor (EDRF), which may be identical to nitric oxide, may occur due to the effect of the atherosclerotic process on endothelial cell function, and may

account for an alteration in vasodilator responsiveness (Kuo et al, 1992). Furthermore, it has been demonstrated in vitro that vasodilatation to exogenous nitrates is enhanced by the removal of endothelium (Shirasaki & Su, 1985), and by inhibition of nitric oxide synthesis (Shirasaki & Su, 1985; Moncada et al, 1991), which appears to be related to an up-regulation of soluble guanylate cyclase (Moncada et al, 1991). If the impairment of the coronary vasodilator reserve after angioplasty was due to reduced production of nitric oxide, then there would be an increase in the vasodilator response with delivery of a high concentration of nitric oxide to the coronary microcirculation. To test this hypothesis in relation to the reduced coronary vasodilator reserve after angioplasty, we have infused high intracoronary doses of the nitric oxide donor, sodium nitroprusside, following intravenous dipyridamole measuring coronary blood flow with a Doppler catheter.

In Chapter 7, the coronary vasodilator reserve in patients with stable single vessel coronary artery disease is investigated as a potential model of resistive vessel dysfunction. Transient myocardial ischaemia in one myocardial region may also lead to an increase in left ventricular dimensions associated with a regional increase in contractility in other remote non-ischaemic regions in animal models (Naccarella et al, 1984; Smalling et al, 1986; Buda et al, 1990) and this may be associated with a regional alteration in myocardial metabolism, such as an increase in glucose and free fatty acid utilisation, and in oxygen consumption (Liedtke et al, 1982). However, it is not known whether patients with recurrent myocardial ischaemia, without evidence of previous infarction or regional wall motion abnormality, may develop alterations in coronary resistive vessel function in the remote regions subtended by angiographically normal coronary arteries as an early abnormality, perhaps due to the atherosclerotic process, as described by Drexler (Drexler et al, 1991) and Zeiher (Zeiher et al, 1991). Thus, resistive vessel dysfunction may occur not only in regions subtended by coronary arteries with proximal disease, but also in regions subtended by normal

arteries in patients with coronary artery disease. To examine coronary resistive vessel function in remote myocardium subtended by angiographically normal arteries, a group of these patients was studied in order to measure both regional coronary vasodilator reserve and metabolic substrate handling in both the region subtended by the stenosis-related artery and a remote region subtended by a normal coronary artery.

In Chapter 8, a model of acute resistive vessel dysfunction is studied where an additional factor such as altered sympathetic nervous system activity is present, limiting further the vasodilator responsiveness of myocardium. This may occur after myocardial infarction with an increase in sympathetic activation over several days even in the absence of significant left ventricular dysfunction. By studying patients with single vessel disease sustaining an uncomplicated myocardial infarction, it is possible to document any changes in resistive vessel function in both the infarcted and remote region in the acute and convalescent phases of infarction. In an animal model of myocardial infarction, a reduced coronary vasodilator reserve has been shown in remote myocardium due to both an increase in basal flow and to reduced vasodilatation, in part attributed to reactive myocyte hypertrophy in the remote myocardium and an increase in end-diastolic pressure (Karam et al, 1990), but associated with reduced basal nitric oxide release several weeks after infarction (Drexler et al, 1992). The mechanism for this reduction in vasodilator responsiveness could perhaps be due to functionally less active endothelium in the resistive vessels, seen after endothelial injury (Shimokawa et al, 1987). In the clinical model of infarction that we have chosen to study, using a PET-derived index of myocardial viability, the perfusable tissue index, it is possible to correlate acute resistive vessel dysfunction with residual viability and regional wall motion using ventriculography .

In Chapter 9, the final model of resistive vessel dysfunction is represented by patients with a blocked coronary artery subtending a non-infarcted territory perfused by collateral vessels from angiographically normal coronary arteries. A recent study

of 11 patients with a single coronary occlusion and collaterals showed that frequent episodes of ST segment depression occurred at heart rates considerably lower than those associated with ST segment depression on exercise testing, and episodes of sinus tachycardia greatly exceeding the rates associated with ST segment depression occurred without such changes (Pupita et al, 1990). The authors suggested that reduced collateral blood flow accounted for these observations and it is possible that constriction of the vessels feeding the collaterals or of the collaterals themselves, or constriction of the (resistive) vessels receiving the collaterals could account for this. As this could account for myocardial ischaemia often documented in these patients, we studied the effect of reflex α-adrenergic stimulation using the cold pressor stress on regional myocardial blood flow and glucose uptake in a group of these patients.

CHAPTER 5. DELAYED RECOVERY OF CORONARY RESISTIVE VESSEL FUNCTION AFTER CORONARY ANGIOPLASTY

5.1 Abstract

The time course of abnormal coronary resistive vessel function in the impairment of the coronary vasodilator reserve was studied after successful coronary angioplasty, using Doppler catheterisation and sequential dynamic positron emission tomography. The coronary vasodilator reserve may be impaired immediately after coronary angioplasty, despite successful dilatation of a flow-limiting stenosis. Twelve male patients (mean age 52±10 years) with single vessel coronary artery disease and normal left ventricular function were studied. The coronary vasodilator reserve to intravenous dipyridamole (0.5 $mg.kg^{-1}$ over 4 minutes) was determined from intracoronary Doppler measurement of coronary flow velocity, before and after successful angioplasty. Basal and maximal myocardial blood flow in the angioplasty region and a normal region were determined in 9 patients using positron emission tomography (PET) with $H_2{}^{15}O$, at 1 day (PET_1), 7 days (PET_2), and 3 months (PET_3) after angioplasty. The coronary vasodilator reserve, measured by Doppler catheterisation, was similar before and immediately after angioplasty, 1.63±0.40 and 1.62±0.55 respectively (p=NS). After angioplasty, in 7 of 9 patients without restenosis, basal myocardial blood flow at PET_1, PET_2, and PET_3, was 0.98±0.16, 0.94±0.09, and 0.99±0.13 $ml.min^{-1}.g^{-1}$ respectively, in the remote region, and 1.19±0.23 ($p<0.01$ vs. remote region), 1.17±0.19 ($p<0.01$ vs. remote region), and 1.10±0.08 $ml.min^{-1}.g^{-1}$ (p=NS vs. remote region) respectively, in the angioplasty region. Myocardial blood flow after dipyridamole at PET_1, PET_2, and PET_3 was 3.04±0.68, 3.00±0.71, and 3.00±0.60 $ml.min^{-1}.g^{-1}$ respectively, in the remote region, and 2.11±0.80 ($p<0.01$

vs. remote region), 2.28±0.73 (p=NS vs. remote region), and 3.06±0.86 $ml.min^{-1}.g^{-1}$ (p=NS vs. remote region) respectively, in the angioplasty region. The coronary vasodilator reserve at PET_1, PET_2, and PET_3 was 3.15±0.85, 3.18±0.68, and 3.08±0.75 respectively, in the remote region, and 1.80±0.68 ($p<0.01$ vs. remote region), 1.94±0.49 ($p<0.01$ vs. remote region), and 2.77±0.74 (p=NS vs. remote region) respectively, in the angioplasty region.

In conclusion, after successful angioplasty, basal myocardial blood flow is increased for at least 7 days in the angioplasty region, with a reduction in the dipyridamole-induced increase in maximal myocardial blood flow for at least 24 hours following the procedure. Thus, the coronary vasodilator reserve is impaired for at least 7 days after angioplasty, indicating that there is abnormal resistive vessel function in the coronary vascular bed distal to a coronary artery stenosis which persists for between 7 days and 3 months.

5.2 Introduction

Animal studies indicate that dilatation of the coronary resistive vessels can allow coronary blood flow to increase 3-4 times above resting values, even in the presence of a 70% diameter stenosis (Gould et al, 1975). However, in patients with coronary artery disease, the behaviour of resistive vessels may be abnormal, and the ischaemic threshold can be altered by changes in vasomotor tone at the level of the resistive vessels rather than in the large epicardial coronary arteries (Pupita et al, 1990). Determination of the contribution of resistive vessel dysfunction to myocardial blood flow limitation is, however, problematic since in the presence of a flow-limiting coronary artery stenosis, it is impossible to separate the effects of altered vasomotion at the site of a stenosis from those of the resistive vessels on modulation of the residual coronary blood flow (Epstein & Talbot, 1981; Maseri et al, 1991).

In order to resolve the difficulty in establishing to what extent coronary blood flow can be modulated by resistive vessel dysfunction, it is necessary to evaluate coronary and regional myocardial blood flow in a clinical model of coronary artery disease which allows exclusion of the effect of a flow-limiting epicardial stenosis, such as following successful percutaneous transluminal coronary angioplasty (PTCA). In addition, as the coronary vasodilator reserve may vary among patients, it is essential to compare, in each case, the behaviour of the vascular bed distal to the stenosis with that of a remote myocardial territory. We used positron emission tomography (PET) to compare the results of Doppler catheterisation of a myocardial region previously subtended by a coronary artery stenosis with a remote region subtended by an angiographically normal artery, and to follow the coronary vasodilator reserve to dipyridamole, non-invasively. We report the changes in basal myocardial blood flow and the myocardial blood flow response to dipyridamole following PTCA, together with the time course of these changes from 1 day up to 3 months after the procedure in diseased and remote myocardial regions.

5.3 Methods

5.3.1. Patient Population

Twelve male patients (mean age 52±10 years, range 39-72) with chronic stable angina were studied. All patients had a positive exercise test. Patients who had suffered a previous myocardial infarction or unstable angina pectoris were excluded. All had proximal left anterior descending coronary artery disease and were undergoing routine PTCA. The left circumflex and right coronary arteries were angiographically normal. Doppler catheterisation was used to evaluate basal and post-dipyridamole coronary blood flow velocity before and immediately after PTCA in 12 patients; in 9 of

these patients, PET was used to evaluate regional basal and post-dipyridamole myocardial blood flow 1 day, 7 days and 3 months after PTCA.

5.3.2. Study Protocol

The protocol was approved by the Research Ethics Committee of Hammersmith Hospital and all patients gave informed and written consent.

All anti-anginal medication (except sublingual nitroglycerin) was discontinued at least 48 hours prior to initial exercise testing and angioplasty, and 72 hours prior to PET scanning. No patient took nitroglycerin within 12 hours of any part of the protocol. Treadmill exercise testing was performed according to the modified Bruce protocol the day before angioplasty. PTCA was performed as clinically indicated. Coronary blood flow velocity and the coronary vasodilator reserve (defined as post-dipyridamole coronary flow velocity/basal coronary flow velocity) (Klocke, 1987), was measured before and after PTCA, as described below. PET scanning with measurement of the myocardial blood flow (MBF) and coronary vasodilator reserve was undertaken 1 day after PTCA (PET_1) in 9 patients (3 patients declined to continue in the study after PTCA). Repeat exercise testing and PET scanning was done at 7±1 days (PET_2) and 100±20 days (PET_3) after PTCA, according to the same protocol as before. In all 9 patients, repeat coronary angiography was performed at 110±25 days.

5.3.2.1. Coronary Blood Flow Velocity Measurement by Doppler Catheterisation

Coronary angiography was performed as clinically indicated in order to best demonstrate the lesion morphology. On completion, an 8-French gauge guiding catheter was inserted into the left coronary ostium. A 0.010 or 0.012 in. "Hi-Torque" guide wire (Advanced Cardiovascular Systems Inc., Santa Clara, Ca.) was advanced across the coronary lesion in the usual fashion. Providing there was no evidence of myocardial ischaemia (chest pain or ECG changes), a 3-French gauge Doppler flow

catheter (Model No. DC 201; Millar Instruments Inc., Houston, Tx., U.S.A.) with a 20 MHz pulsed Doppler crystal, referenced to zero and calibrated, was advanced over the guide wire. The tip of the Doppler catheter was positioned up to 5 mm proximal to the stenosis (Wilson et al, 1985). The Doppler velocimeter range control was adjusted to obtain an optimal audio signal and both phasic and mean tracing of maximal resting coronary blood flow velocity.

Coronary flow velocity, heart rate and systemic arterial pressure were measured continuously. Resistive vessel dilatation was induced by dipyridamole (0.5 $mg.kg^{-1}$ intravenously over 4 minutes). In 7 patients, coronary flow velocity was measured before PTCA. PTCA was then performed entirely as clinically indicated using an A.C.S. RX balloon angioplasty catheter (Advanced Cardiovascular Systems Inc., Santa Clara, Ca., U.S.A). Following the procedure, the angioplasty catheter was removed leaving the guide wire in situ. The Doppler flow catheter was re-positioned as described above. Repeat measurements of basal and maximal coronary blood flow velocity after dipyridamole were performed as described above in all 12 patients.

5.3.2.2. Quantitative Coronary Arteriography

Coronary arteriograms were analysed by an automated edge contour detection computer analysis system (Cardiovascular Angiographic Analysis System [CAAS], Pie Medical Equipment BV, Maastricht, The Netherlands) (Reiber et al, 1984). The luminal diameter of the coronary stenosis in the projection showing maximum severity, and of the proximal reference segment, were measured at end-diastole with care taken to select a view free from overlapping vessels. This was done under basal conditions and at the peak effect on coronary flow velocity of dipyridamole both before and after angioplasty. The diameter of the stem of the Judkins coronary catheter was used for calibration in order to obtain measurements in absolute units (mm). Correction was made for radiographic pincushion distortion. Stenosis severity

was also expressed as percentage reduction of the internal luminal diameter relative to the angiographically normal proximal coronary segment, the reference segment. Restenosis was defined using the same criteria as Beatt et al (Beatt et al, 1992): a reduction in the residual lumen diameter of ≥0.72 mm and ≥50% diameter stenosis.

The coronary vascular resistance index was calculated as the quotient of mean arterial pressure and coronary blood flow velocity, and expressed as units (Kern et al, 1989).

5.3.2.3. Regional Myocardial Blood Flow Measurement with PET

Regional myocardial blood flow (MBF) was measured using 15-oxygen (^{15}O) and dynamic PET (Araujo et al, 1991). This was performed at three time points after coronary angioplasty in 9 patients: i) within 24 hours, ii) 7±1 days, and iii) 100±20 days. All PET scans were recorded with an ECAT 931-08/12 scanner (CTI Inc., Knoxville, Tn., U.S.A.). The scanner consists of 8 contiguous rings of bismuth germanate detectors which allows 15 cross-sectional images of the heart to be visualised simultaneously in a 10.5 cm axial field of view. All emission scans were reconstructed with a Hanning filter which had a cut-off frequency of half maximum. This resulted in a transaxial resolution of 8.4±0.7 mm FWHM (full width at half maximum) for the emission data at the centre of the field of view (Spinks et al, 1988). Thus, it was possible to record simultaneously myocardial and blood tracer concentrations of the whole heart (Chapter 3, Section 3.4).

1. Scanning procedure. Patients abstained from drinking tea or coffee on the morning of the scan and all had been off anti-anginal medication for at least 72 hours. After PTCA, patients were treated with intravenous nitrate until 12 hours prior to the scan.

Patients were positioned in the scanner and a 5 minute rectilinear transmission scan was recorded to allow positioning of the left ventricle in the centre of the field of view. This was done by exposing a circular ring source using 2 mCi of germanium-68. Another transmission scan was then recorded for 20 minutes which was used to correct all emission scans for tissue attenuation. The patient's position with respect to the camera was determined by a cross-shaped low power laser beam and pen marks on the patient's skin. Following transmission, radioactive gases were continuously delivered by an MC face-mask. Blood pool scans were performed with ^{15}O-labelled carbon monoxide ($C^{15}O$). This tracer, which forms ^{15}O-carboxyhaemoglobin, was continuously delivered at at a flow rate of 500ml·min^{-1} (3 MBq·ml^{-1}) for 4 minutes, and a 6 minute single frame scan was recorded starting one minute after the end of gas delivery. Venous blood samples were taken before and after gas delivery. The $C^{15}O$ concentration was measured with a sodium iodide well counter cross-calibrated with the scanner. After a 10 minute period to allow for decay, a continuous inhalation of $C^{15}O_2$ was given for 3.5 minutes (4 MBq.ml^{-1} at a flow rate of 500 ml·min^{-1}). The $C^{15}O_2$ is converted immediately by the enzyme carbonic anhydrase in the lung capillaries to ^{15}O-labelled water ($H_2{}^{15}O$), which is delivered to pulmonary venous blood (Clark & Buckingham, 1975). A sequence of 24 scans started with $C^{15}O_2$ delivery, consisting of 6 scans of 5, 10, 20, and 30 seconds. This produced a build-up scan over 3.5 minutes and a washout scan over 3 minutes. Basal MBF was measured in all patients. After 10 minutes to allow for decay, the myocardial blood flow was measured following an intravenous infusion of dipyridamole (0.5 mg·kg^{-1} over 4 minutes). The delivery of $C^{15}O_2$ started two minutes after the end of this infusion. A standard 12 lead electrocardiogram was recorded every minute during the dipyridamole infusion and for up to 8 minutes after the infusion. Blood pressure was measured every minute by a cuff sphygmomanometer during the same period of time.

2. Analysis of PET data. The sinograms were corrected for attenuation and reconstructed on a Microvax II computer (Digital Equipment Corporation, Marlboro, Ma.) using dedicated array processors and standard reconstruction algorithms (Araujo et al, 1991; Spinks et al, 1988). Images were transferred to a SUN 3/60 work-station for further analysis using Analyze™ (Mayo Foundation, Rochester, Mn.,U.S.A.) and Pro-Matlab™ (The Mathworks Inc., South Natick, Ma., U.S.A.) software packages.

A blood volume image was created using the $C^{15}O_2$ data, by dividing the raw image by the product of the average venous blood radioactivity and the density of whole blood (1.06 $g \cdot ml^{-1}$). Either 4 or 5 transaxial regions of interest were drawn within the left atrium which were projected onto the dynamic ^{15}O-water images to generate time-activity curves for each region. The average was used as arterial input function. Images of extravascular volume were created by subtracting blood volume from transmission images after the latter were normalized for tissue density (1.04 $g \cdot ml^{-1}$). In addition, the blood volume images were subtracted from the integrated time frames of the washout phase of the ^{15}O-water studies. The images of extravascular volume and extravascular ^{15}O-water were used to delineate 4 regions of myocardium (anterior, lateral, infero-posterior and septal) over 5-7 transaxial planes, and data averaged prior to the modelling of myocardial perfusion (Figure 5.1). The regions of interest were superimposed onto the kinetic time frames recorded during the $C^{15}O_2$ inhalation and washout. This generated myocardial tissue time-activity curves for each region, which together with arterial time-activity curves, were fitted to a single tissue compartment tracer kinetic model to give values for regional MBF (in $ml \cdot min^{-1} \cdot g^{-1}$) (Araujo et al, 1991).

In all patients, the anterior region was designated as the PTCA region and the infero-posterior region was designated as the remote region. Basal MBF and MBF after dipyridamole were determined in both regions. The coronary vasodilator reserve was defined as post-dipyridamole MBF/ basal MBF (Klocke, 1987).

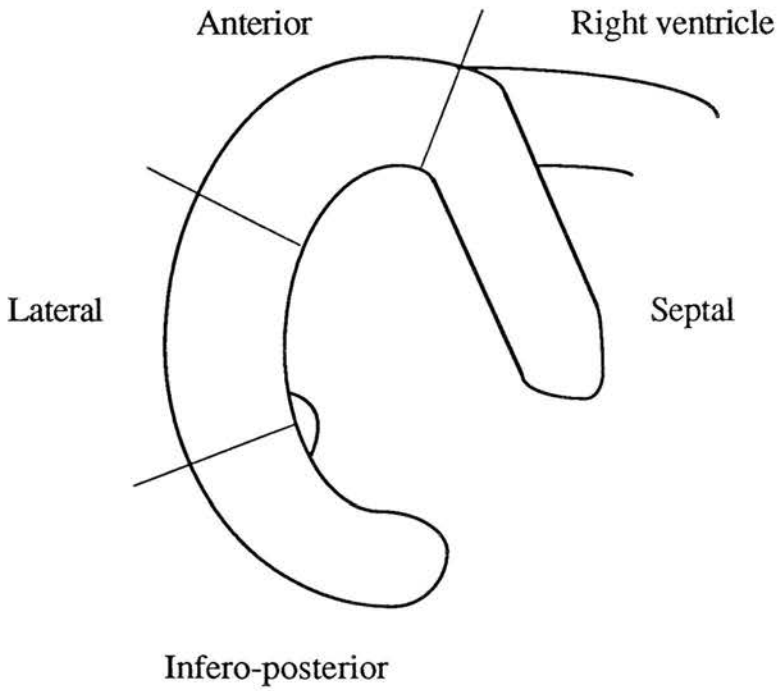


Figure 5.1. A schematic of the standard regions of interest drawn on the left ventricular extravascular density image and transposed to the washout image. The anterior region was drawn from the intersection of right ventricular free wall with septum. The demarcation between lateral and inferoposterior regions was drawn at the level of the posterior papillary muscle.

5.3.3. Statistical Analysis

All values are expressed as mean±SD. Data from Doppler catheterisation before and after angioplasty was analysed using the paired Students t test. Two-way analysis of variance (ANOVA) was used to compare data derived from PET scanning at the different time intervals. Specific comparisons were done using the paired Students t test corrected for multiple comparisons with the Boneferroni inequality adjustment (Wallenstein et al, 1980). Linear regression analysis was used to examine the relationship between coronary artery dimensions and the the coronary vasodilator reserve measured by Doppler. MBF values in the regions of interest in each individual plane for each scan were derived and the coefficient of variation within and between groups measured by one-way ANOVA. A p value <0.05 was considered statistically significant.

5.4 Results

Doppler catheterisation was performed in 7 patients before PTCA, and in all 12 patients after PTCA (Table 5.2). PET scanning was done 1 day after PTCA in 9 patients (3 patients declined to continue in the study after PTCA). Seven (7±1) days and 3 months (100±20 days) after PTCA, the same 9 patients underwent repeat exercise testing and PET scanning, according to the same protocol as before (Tables 5.4 and 5.5). In all 9 patients, repeat coronary angiography was done 110±25 days after the initial PTCA. Two patients who had developed recurrent angina and who had a positive exercise test at this time, had restenosis of the dilated arterial segment, as defined above.

5.4.1. Exercise Testing/

5.4.1. Exercise Testing

(Figure 5.2)

All patients underwent treadmill exercise testing at least 48 hours off all anti-anginal medication before PTCA (Figure 5.2). Nine patients agreed to continue in the study after PTCA. Before PTCA, the rate-pressure product (RPP) was 9,160±2,880 mmHg·min^{-1} at rest, 18,310±7,820 mmHg·min^{-1} at 0.1mV ST segment depression, and of 20,750±7,670 mmHg.min^{-1} at peak exercise. Seven days post-PTCA, all 9 patients performed negative tests; the RPP at peak exercise was 29,410±7,920 mmHg·min^{-1} ($p<0.05$ vs. peak RPP pre-PTCA). In the 7 patients with no restenosis, the RPP at peak exercise was 20,960±7,830 mmHg·min^{-1} pre-PTCA, and 29,550±8,690 mmHg·min^{-1} at 7 days ($p<0.05$ vs. peak RPP pre-PTCA) (Figure 5.2). All 9 patients underwent exercise testing approximately 3 months (100±20 days) after PTCA. The exercise tests were negative in 6 patients and positive in 3 patients. Of these 3 patients, 2 had restenosis: 1 asymptomatic patient without restenosis (patient 3) reached 0.1mV ST segment depression at a RPP of 25,830 mmHg.min^{-1} (compared to 8370 mmHg·min^{-1} pre-PTCA), unassociated with symptoms of chest pain. Thus, the RPP at peak exercise in the 7 patients without restenosis was 29,560±9,310 mmHg·min^{-1} (Figure 5.2).

In the 2 cases with restenosis at 3 months, both had negative tests at 7 days. Patient 8 had an RPP to 0.1mV ST segment depression of 18,600 mmHg.min^{-1} at 3 months (cf. 9,960 mmHg·min^{-1} pre-PTCA), and patient 9 had an RPP to 0.1mV ST segment depression of 21,450 mmHg·min^{-1} at 3 months (cf. 21,250 mmHg·min^{-1} pre-PTCA) (Figure 5.2).

5.4.2. Quantitative Coronary Arteriography/

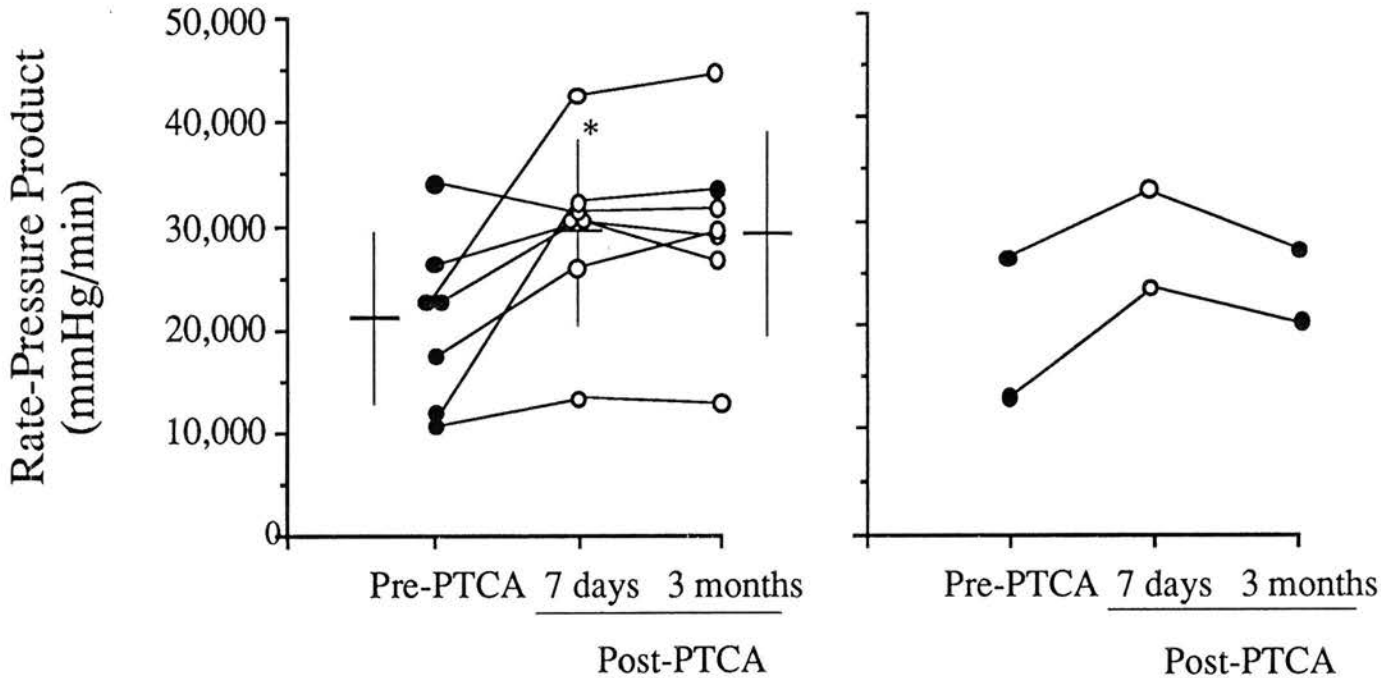


Figure 5.2. Exercise testing before, at 7 days and at 100 days after angioplasty in patients without (n=7) (left panel) and with restenosis (n=2) (right panel). (* $p<0.05$ vs. pre-PTCA). No significant ST depression (open symbols), ≥0.1 mV ST segment depression (black symbols).

5.4.2. Quantitative Coronary Arteriography

(Table 5.1)

The results of quantitative coronary arteriography are shown in Table 5.1. There was an increase in luminal diameter at the site of stenosis from 0.64±0.28 mm to 1.86±0.48 mm ($p<0.0001$) immediately after PTCA, corresponding to a reduction in percentage luminal stenosis severity from 73.1±10.3% to 26.2±7.2% ($p<0.0001$). Pre-PTCA, at the peak effect of dipyridamole, there was no significant change in diameter at the stenosis or of the reference segment, 0.74±0.27 and 2.29±0.38 mm, respectively. Post-PTCA, there was no significant change in diameter at the stenosis, 1.86±0.48 and 1.84±0.46 mm, or of the reference segment, 2.50±0.50 and 2.54±0.39 mm, from basal to post-dipyridamole, respectively.

At follow-up arteriography, there was no evidence of restenosis in 7 patients, with a lumen diameter at the site of stenosis of 1.80±0.23 mm (p=NS vs. post-PTCA stenosis diameter) and a reference diameter of 2.61±0.36 mm (p=NS vs. post-PTCA reference diameter). In 2 cases, there was evidence of restenosis: a residual lumen diameter of 0.46 mm (reference diameter of 3.11 mm) in patient 8 and complete occlusion at the site of previous PTCA in patient 9. In patient 9, there was no clinical or ECG evidence of myocardial infarction.

5.4.3. Coronary Flow Velocity with Doppler Catheterisation

(Table 5.2, Figures 5.3 and 5.4)

Seven patients underwent Doppler catheterisation before PTCA (Table 5.2). Heart rate increased from 64±8 beats·min^{-1} at basal to 82±5 beats·min^{-1} at the peak effect of dipyridamole ($p<0.01$), with no significant reduction in systolic (149±18 to 140±15 mmHg), diastolic (92±9 to 87±10 mmHg) or mean (111±11 to 106±11 mmHg) arterial pressure. Coronary flow velocity increased from a basal value of 7.5±3.9 cm·s^{-1}, to 11.3±3.6 cm·s^{-1} ($p<0.01$) after dipyridamole, with a coronary vasodilator

Table 5.1. Quantitative Coronary Arteriographic Findings in 12 Patients

	Basal State	Peak Dipyridamole Effect
Before angioplasty		
Stenosis (mm)	0.64±0.28	0.74±0.27
Reference (mm)	2.30±0.37	2.29±0.38
% stenosis	73.1±10.3%	67.6±8.9%
After angioplasty		
Stenosis (mm)	1.86±0.48 *	1.84±0.46 *
Reference (mm)	2.50±0.50	2.54±0.39
% stenosis	26.2±7.2% *	27.8±13.1% *
Follow-up arteriography†		
Stenosis (mm)	1.80±0.23	-
Reference (mm)	2.61±0.36	-
% stenosis	29.6±16.0%	-

* $p<0.0001$ vs. value before PTCA. † Data are from the 7 patients without restenosis among the 9 patients who underwent follow-up arteriography.

reserve of 1.63±0.40 (Figure 5.3). The coronary vascular resistance index fell from 17.5±6.4 units at basal to 10.1±3.2 units ($p<0.01$) with dipyridamole (Figure 5.4).

Twelve patients underwent Doppler catheterisation after PTCA (Table 5.2). Heart rate increased from 66±13 beats·min^{-1} at basal to 81±18 beats·min^{-1} at the peak effect of dipyridamole ($p<0.001$), with no significant reduction in systolic (135±21 mmHg to 130±20 mmHg), diastolic (94±10 to 87±12 mmHg) or mean (100±12 to 96±15 mmHg) arterial pressure. The basal coronary flow velocity was 9.2±5.0 cm·s^{-1} and the maximal coronary flow velocity after dipyridamole was 13.8±5.7 cm·s^{-1} ($p<0.001$ vs. basal). The coronary vascular resistance index fell from 13.2±5.2 units at basal to 7.8±2.8 units with dipyridamole ($p<0.01$ vs. basal), with a coronary vasodilator reserve of 1.62±0.48. With linear regression analysis, no relationship was demonstrated between residual stenosis diameter after angioplasty and the coronary vasodilator reserve.

Coronary flow velocity and coronary vascular resistance index were compared in the 7 patients who were studied before and after PTCA. In these 7 patients, the mean time between the start of each dipyridamole infusion was 55.6±10.6 minutes. There were no significant differences in systolic, diastolic or mean arterial pressures at basal or at peak dipyridamole before or after PTCA. Post-PTCA, the basal coronary blood flow velocity was significantly increased to 9.9±6.2 cm·s^{-1} compared with pre-PTCA ($p<0.05$ vs. pre-PTCA basal), although that after dipyridamole, 14.5±6.9 cm·s^{-1}, was not significantly changed (p=NS vs. pre-PTCA peak dipyridamole) (Figure 5.3). Post-PTCA, the coronary vasodilator reserve was 1.62±0.55 (p=NS vs. pre-PTCA). The basal coronary vascular resistance index was significantly lower post-PTCA, 13.6±6.2 units ($p<0.05$ vs. pre-PTCA basal), although the value after dipyridamole, 8.3±3.1 units, was similar to that pre-PTCA (p=NS vs. pre-PTCA peak dipyridamole) (Figure 5.4).

Table 5.2. Doppler Coronary Flow Velocity Before and After Angioplasty

	Before Angioplasty (n=7)			After Angioplasty (n=12)		
Pts	Basal State	Peak Dipyridamole Effect	Coronary Vasodilator Reserve	Basal State	Peak Dipyridamole Effect	Coronary Vasodilator Reserve
1	4.3	9.2	2.14	4.0	11.0	2.75
2	15.5	15.5	1.00	22.0	27.0	1.23
3	-	-	-	12.0	15.0	1.25
4	-	-	-	6	7.5	1.25
5	7.0	12.0	1.71	7.5	9.0	1.20
6	4.6	6.8	1.48	6.0	8.8	1.47
7	5.9	12.2	2.07	7.4	14.3	1.93
8	-	-	-	10.0	17.8	1.78
9	-	-	-	7.0	11.2	1.6
10	-	-	-	5.6	12.5	2.23
11	5.8	7.6	1.31	8.0	10.8	1.35
12	9.2	15.8	1.72	14.6	20.8	1.42
Mean ±SD	7.5±3.9	11.3±3.6 *	1.63±0.41	9.2±5.0 §	13.8±5.7 †	1.62±0.48

* $p<0.01$ vs. Basal, † $p<0.001$ vs. Basal; § $p<0.05$ vs. before angioplasty. All flow values in $cm \cdot s^{-1}$.

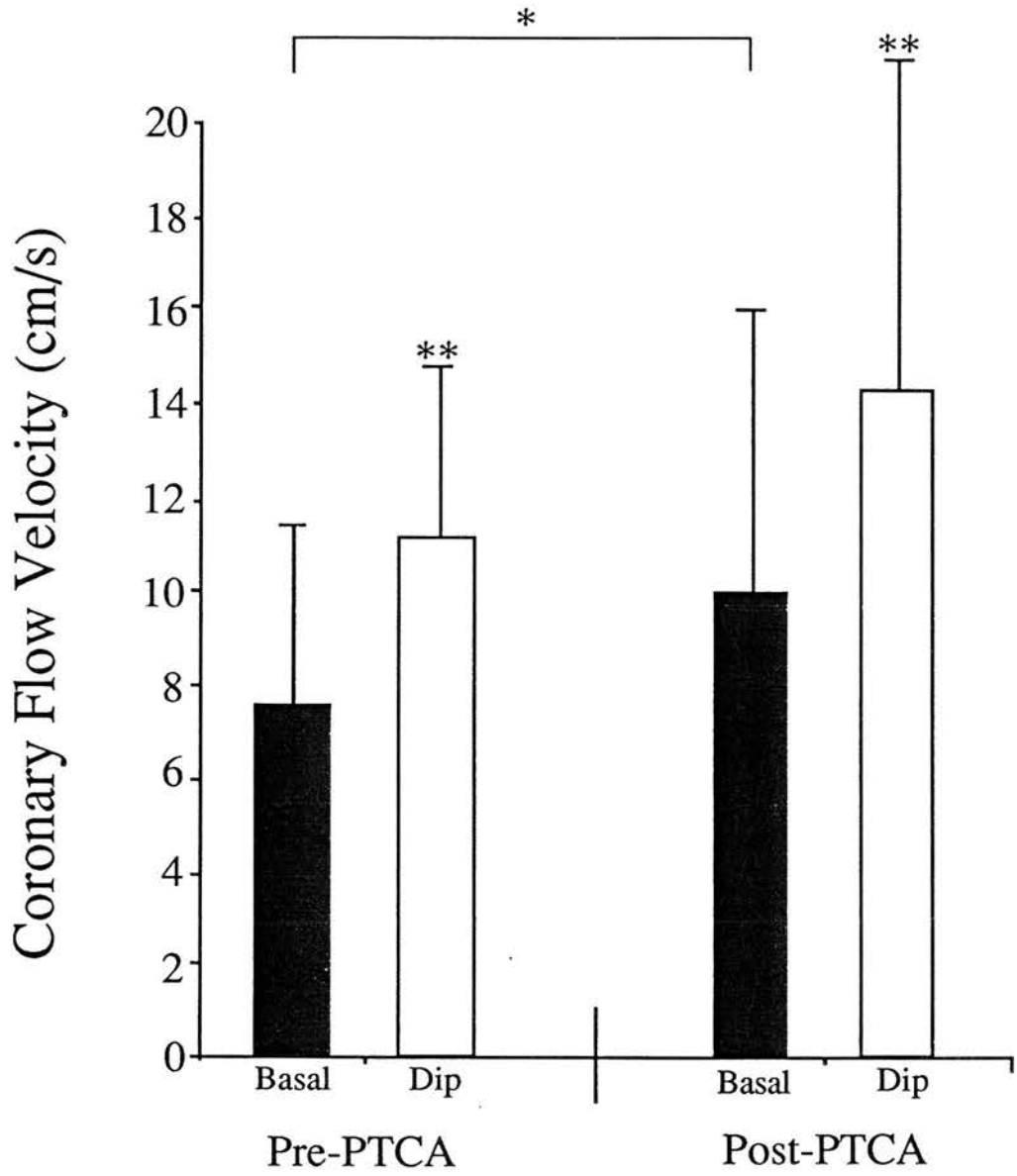


Figure 5.3. Bar graphs showing coronary flow velocity at basal and at peak dipyridamole (Dip) before PTCA (n=7) and after PTCA (n=7). (* $p<0.05$ vs. pre-PTCA. ** $p<0.01$ vs. Basal).

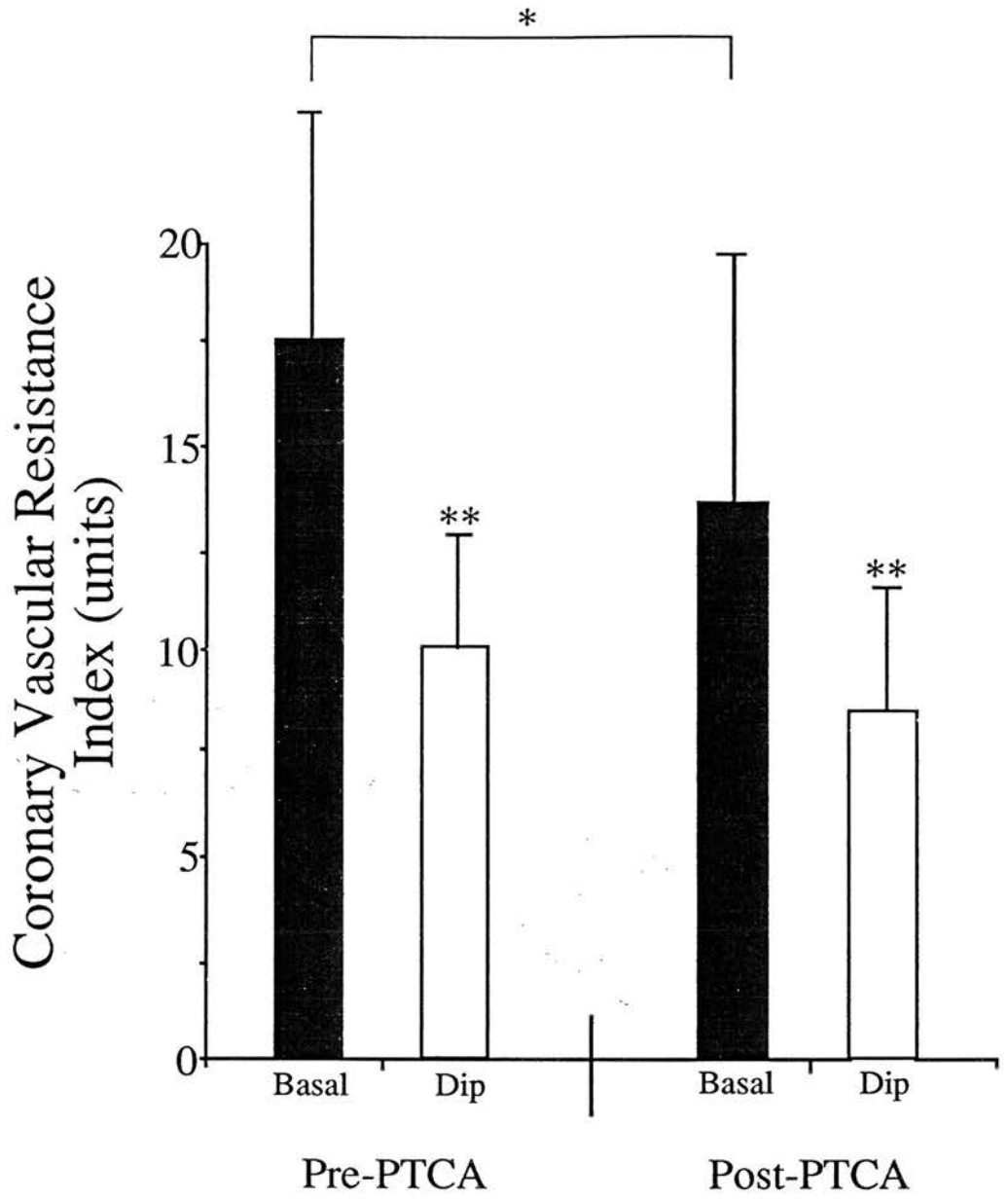


Figure 5.4. Bar graphs showing coronary vascular resistance index at basal and at peak dipyridamole (Dip) before PTCA (n=7) and after PTCA (n=7). (* $p<0.05$ vs. pre-PTCA, ** $p<0.01$ vs. Basal).

5.4.4. Regional Myocardial Blood Flow with PET

(Tables 5.3 to 5.5, Figures 5.5 and 5.6)

Heart rate, systolic and diastolic blood pressure, and rate-pressure product at basal and after dipyridamole at each PET study are shown in Table 5.3. There were no significant differences in these haemodynamic parameters comparing all three studies. Individual myocardial blood flow (MBF) at basal and after dipyridamole and the coronary vasodilator reserve at 1 day, 7 days and 3 months are shown in Tables 5.4 and 5.5.

One day after PTCA, in the 7 of 9 patients without restenosis at three months, MBF at rest was significantly higher in the PTCA region, 1.19±0.23 $ml \cdot min^{-1} \cdot g^{-1}$, compared to the remote region, 0.98±0.16 $ml \cdot min^{-1} \cdot g^{-1}$ ($p<0.01$) (Figure 5.5). This increase in basal MBF was still present at 7 days after PTCA, 1.17±0.19 $ml \cdot min^{-1} \cdot g^{-1}$ in the PTCA region compared with 0.94±0.09 $ml \cdot min^{-1} \cdot g^{-1}$ in the remote region ($p<0.01$). Three months after PTCA, there was no significant difference between basal MBF in the PTCA and remote regions (1.10±0.08 and 0.99±0.13 $ml \cdot min^{-1} \cdot g^{-1}$, respectively) (Table 5.4 and Figure 5.5).

The dipyridamole-induced increase in MBF in the PTCA region at 1 day, 2.11±0.80 $ml \cdot min^{-1} \cdot g^{-1}$, was lower than the remote region, 3.04±0.68 $ml \cdot min^{-1} \cdot g^{-1}$ ($p<0.01$). At 7 days, although the MBF response to dipyridamole was still lower in the PTCA region compared to the remote region (2.28±0.73 and 3.00±0.71 $ml \cdot min^{-1} \cdot g^{-1}$, respectively), this was not a significant difference. At 3 months after PTCA, the MBF response to dipyridamole was similar in the PTCA region and the remote region (3.06±0.86 and 3.00±60 $ml \cdot min^{-1} \cdot g^{-1}$, respectively) (Table 5.4 and Figure 5.5).

One day after PTCA, the coronary vasodilator reserve was significantly lower in the PTCA region, 1.80±0.68, compared with the remote region, 3.15±0.85 ($p<0.01$) (Figure 5.6). This difference was present 7 days after PTCA; 1.94±0.49 in the PTCA region, compared to 3.18±0.68 in the remote region ($p<0.01$). Three months after

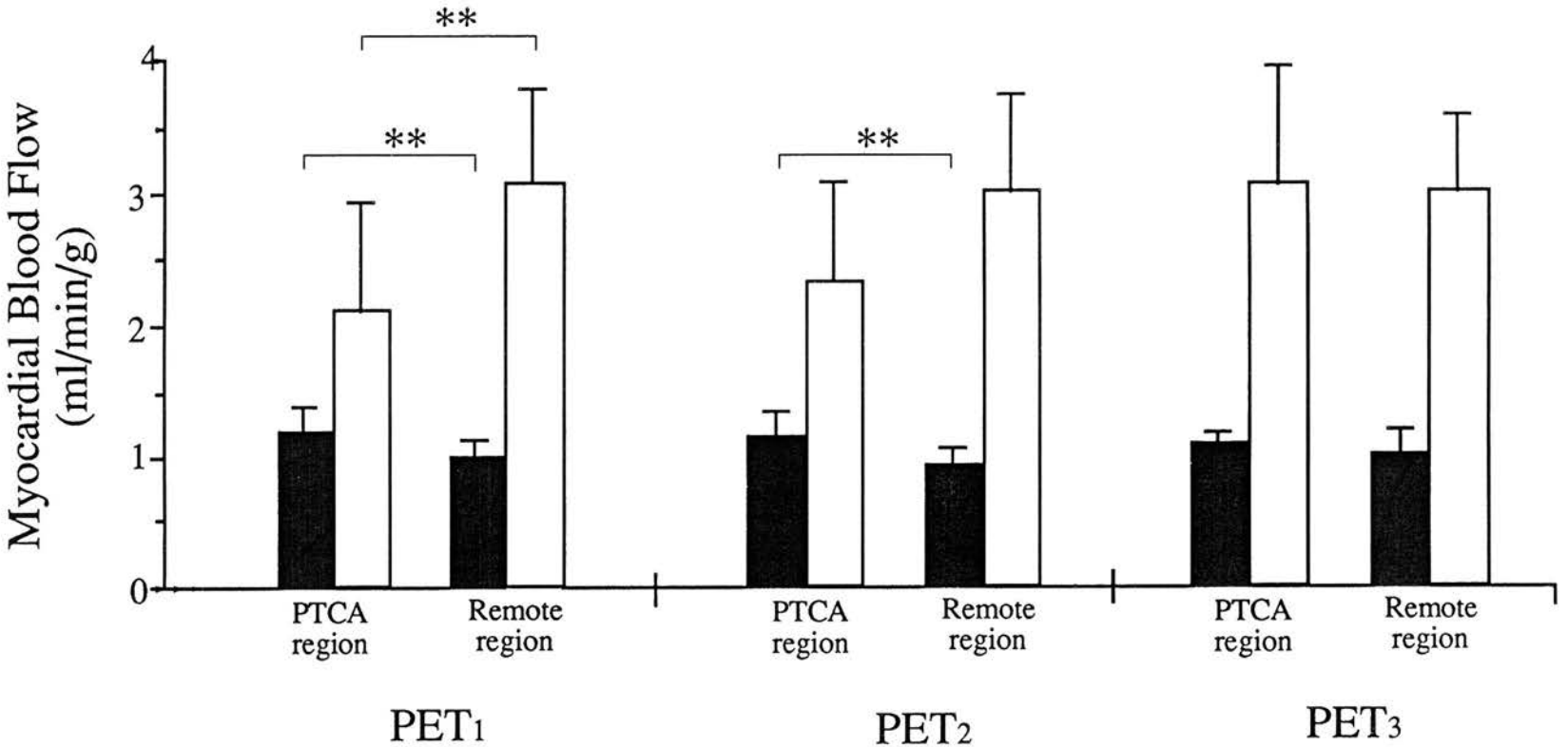


Figure 5.5. Bar graphs showing myocardial blood flow at basal and after dipyridamole in the PTCA and the remote myocardial regions at PET1 (1 day), PET2 (7 days), and PET3 (3 months) after successful PTCA. Basal state = solid bar, peak dipyridamole effect = open bar. ** $p<0.01$.

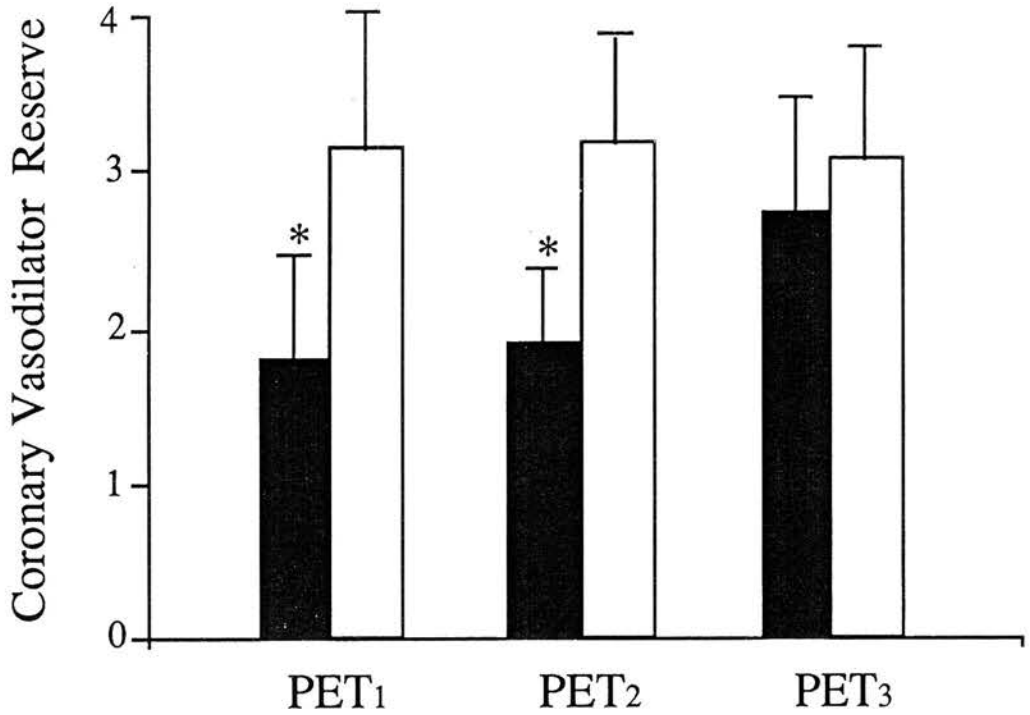


Figure 5.6. Bar graphs showing the coronary vasodilator reserve in the angioplasty and the remote myocardial regions at PET1 (1 day), PET2 (7 days), and PET3 (3 months) after successful angioplasty. Angioplasty region = solid bar, remote region = open bar. * $p<0.05$.

Table 5.3. Haemodynamic Variables at Positron Emission Tomography

	Basal State				Peak Dipyridamole Effect			
Study	Heart Rate (beats·min^{-1})	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Rate-Pressure Product (mmHg·min^{-1})	Heart Rate (beats·min^{-1})	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Rate-Pressure Product (mmHg·min^{-1})
PET_1	63±12	120±11	73±13	7,590±1,440	78±16	127±19	75±13	9,860±2,550
PET_2	61±9	123±10	76±10	7,590±1,460	79±13	128±14	76±8	10,190±2,480
PET_3	66±7	120±14	80±7	7,860±1,380	82±10	130±13	78±8	10,890±2,110

PET_1, PET_2, PET_3 = PET scan at 1 day, 7 days and 100 days, respectively, after angioplasty.

Table 5.4. Regional Myocardial Blood Flow at Positron Emission Tomography

	PET_1				PET_2				PET_3			
	PTCA Region		**Remote Region**		**PTCA Region**		**Remote Region**		**PTCA Region**		**Remote Region**	
Pts.	Basal	Dip	Basal	Dip	Basal	Dip	Basal	Dip	Basal	Dip	Basal	Dip
1	1.24	1.43	1.14	3.01	1.46	3.54	1.11	2.81	1.22	3.90	0.89	3.38
2	1.06	1.38	0.92	1.87	0.97	1.36	0.80	1.92	1.04	1.63	0.78	2.10
3	0.84	2.14	0.74	3.22	1.07	2.65	0.98	3.93	1.01	3.72	0.96	3.89
4	1.20	1.45	0.85	3.48	1.29	1.68	0.96	3.63	1.05	3.38	0.95	2.97
5	1.19	2.05	1.01	2.59	1.00	1.80	0.92	2.38	1.04	2.15	1.14	2.40
6	1.60	2.81	1.08	3.11	1.06	2.50	0.90	3.40	1.19	3.03	0.87	3.09
7	1.21	3.50	1.15	4.02	1.33	2.43	0.94	2.96	1.15	3.58	1.20	3.15
Mean ±SD	1.19±0.23 *	2.11±0.80 *	0.98±0.16	3.04±0.68	1.17±0.19 *	2.28±0.73	0.94±0.09	3.00±0.71	1.10±0.08	3.06±0.86	0.99±0.13	3.00±0.60
8	1.46	1.86	0.74	2.33	0.9	1.42	0.79	1.19	0.82	1.05	0.84	0.91
9	1.60	1.81	1.00	3.61	1.10	1.34	0.79	2.78	0.78	1.48	0.69	1.26

* $p<0.01$ vs. remote region, 1 day (PET_1), 7 days (PET_2), and 100 days (PET_3) after successful coronary angioplasty in the 7 patients without restenosis at follow-up angiography. The individual values for the two patients with restenosis are given for comparison. Basal = basal conditions; Dip = peak dipyridamole effect; other abbreviations as in Table 5.3.

Table 5.5. Coronary Vasodilator Reserve at Positron Emission Tomography

	PET_1		PET_2		PET_3	
Patients	PTCA Region	Remote Region	PTCA Region	Remote Region	PTCA Region	Remote Region
1	1.15	2.64	2.42	2.53	3.20	3.80
2	1.30	2.03	1.40	2.40	1.57	2.36
3	2.55	4.35	2.48	4.01	3.68	4.05
4	1.21	4.09	1.30	3.78	3.22	3.11
5	1.72	2.56	1.80	2.59	2.07	2.11
6	1.76	2.88	2.36	3.78	2.55	3.55
7	2.89	3.50	1.83	3.15	3.11	2.62
Mean±SD	1.80±0.68 *	3.15±0.85	1.94±0.49 *	3.18±0.68	2.77±0.74	3.08±0.75
8	1.27	3.01	1.58	1.51	1.28	1.08
9	1.13	3.61	1.22	3.52	1.90	1.83

* $p<0.01$ vs. remote region. Individual coronary vasodilator reserves in the PTCA and remote regions, 1 day (PET_1), 7 days (PET_2), and 100 days (PET_3) after successful coronary angioplasty in the 7 patients without restenosis at follow-up angiography. The individual values for the two patients with restenosis are given for comparison.

PTCA, the coronary vasodilator reserve was similar in the two regions; 2.77±0.74 and 3.08±0.75 (p=NS) in the PTCA and remote regions, respectively.

In the 2 patients with restenosis at 3 months, there was an impaired coronary vasodilator reserve in the PTCA region (1.28 in patient 8 and 1.90 in patient 9) compared to the mean value of 2.77±0.74 in the 7 patients without restenosis. Of interest, in the remote region, the coronary vasodilator reserve was also impaired (1.08 in patient 8 and 1.83 in patient 9) compared to the mean value of 3.08±0.75 in the 7 patients without restenosis (Tables 5.4 and 5.5).

To assess variability in the MBF measurements in the PTCA and remote regions in individual planes at each scan, the coefficient of variation was measured. At basal, the coefficient of variation in the PTCA region was 21.3±9.7%, 23.1±12.6%, and 22.3±9.4% (all p=NS), and in the remote region was 24.9±10.6%, 30.4±9.8% and 28.7±3.7% (all p=NS) at PET_1, PET_2, and PET_3, respectively. After dipyridamole, the coefficient of variation in the PTCA region was 23.8±8.7%, 25.9±10.8%, and 24.7±12.2% (all p=NS), and in the remote region was 30.5.±10.0%, 33.8±5.5% and 36.1±10.1% (all p=NS) at PET_1, PET_2, and PET_3, respectively. These coefficients of variation are similar to that reported previously (Araujo et al, 1991).

5.5 Discussion

This study shows that immediately following successful coronary angioplasty, despite a marked and significant reduction in stenosis severity, maximal coronary blood flow velocity following dipyridamole was similar to that observed before angioplasty, and the coronary vasodilator reserve remained unchanged. Basal coronary blood flow velocity in the region subtended by the stenosed artery before angioplasty was similar to that reported in normals (Wilson et al, 1985), and increased immediately after the angioplasty. Measurement of basal myocardial blood flow

showed that the increase persisted for at least 7 days in the angioplasty region after PTCA, compared with the remote region, but by three months, it had returned to normal. The maximal myocardial blood flow response to dipyridamole in the angioplasty region was lower than that in the remote region 24 hours after angioplasty, consistent with the observations made immediately post-PTCA with the Doppler catheter, but by 7 days it had returned towards normal values. Thus, the coronary vasodilator reserve in the region subtended by the previously stenosed coronary artery is impaired for the first 24 hours after angioplasty due to an increase in basal coronary blood flow and a reduction in the maximal vasodilator response. At 7 days, it is still impaired due to an increase in basal coronary blood flow although the maximal blood flow response to dipyridamole has returned towards normal.

Persistent Reduction in the Coronary Vasodilator Response after Angioplasty

We were able to document a reduced coronary vasodilator reserve both by Doppler catheterisation and by PET. The PET study allowed us to show that the coronary vasodilator reserve was significantly lower in the angioplasty region than in the remote region for at least one week after successful angioplasty. The dipyridamole infusion induced an increase in myocardial blood flow and a coronary vasodilator reserve in the remote region similar to that previously described in patients with chronic stable angina and single vessel coronary artery disease, although lower than the value usually reported in normal subjects (Araujo et al, 1991). We (Uren et al, 1993b) and others (Sambuceti et al, 1991; Beanlands et al, 1992), have shown that the coronary vasodilator reserve to dipyridamole is lower in myocardium supplied by angiographically normal arteries in patients with single vessel coronary artery disease and normal left ventricles, compared with patients without coronary artery disease (Chapter 7). The value that we found in the remote region in the present study is

similar to that reported before in patients with single vessel disease (Araujo et al, 1991).

Alleviation of the flow-limiting coronary artery stenosis would be expected to permit a larger coronary blood flow when resistive vessel dilatation is induced. Our findings thus indicate that there was a clear failure of the resistive vessels to dilate in response to dipyridamole within the first 24 hours. Furthermore, post-PTCA, there may have been residual effects of the first dose of dipyridamole on myocardial blood flow and this might be expected to augment the response to the second dose of dipyridamole. Thus, a possible explanation for the higher basal blood flow post-PTCA is that it was caused by the residual effects of the first dose of dipyridamole. This seems an unlikely explanation however since basal myocardial blood flow measured by PET revealed a higher value in the PTCA region compared with the remote region for at least the first 7 days post-PTCA. A more striking finding was that the maximal increase in blood flow velocity in response to dipyridamole post-PTCA was not increased compared to pre-PTCA, clearly indicating a failure of resistive vessel dilatation.

Comparison with Other Studies

Several groups have described an impaired coronary vasodilator reserve after PTCA (Kern et al, 1989; Wilson et al, 1988; Zijlstra et al, 1988; Nanto et al, 1992). Wilson et al. (Wilson et al, 1988) reported that the coronary vasodilator reserve to papaverine, measured by Doppler catheter, was impaired immediately after angioplasty in 55% of patients, and that in these patients, it had returned to normal by a mean follow-up period of 7.5 months. Zijlstra et al (Zijlstra et al, 1988) reported that the coronary vasodilator reserve to papaverine was similarly impaired in all their patients immediately after PTCA. Neither of these studies reported either basal or peak coronary blood flow or velocity, but only the coronary vasodilator reserve. Kern et al.

(Kern et al, 1989) and Nanto et al. (Nanto et al, 1992) reported an impaired coronary vasodilator reserve immediately post-PTCA due to both an increase in basal coronary blood flow and a decreased maximal coronary blood flow response to papaverine and atrial pacing respectively. These reports are consistent with our study; basal coronary blood flow was increased immediately post-PTCA and thereafter for at least 7 days, and the maximal coronary blood flow response to dipyridamole was impaired immediately post-PTCA and for at least the first 24 hours. The coronary vasodilator reserve was therefore reduced for at least the first 7 days post-PTCA. Laarman et al (Laarman et al, 1991) reported no change in the coronary vasodilator reserve to papaverine using digital subtraction angiography immediately after PTCA and, as in our study, no correlation was shown between residual stenosis diameter and the impaired coronary vasodilator reserve.

Walsh et al (Walsh et al, 1990) used PET to measure the coronary vasodilator reserve after angioplasty, and reported that it returned to normal in all patients following the procedure, but this study was limited since individual patients were studied at different time intervals after angioplasty, ranging from 1 to 18 days (a mean of 11 days) with no serial studies. It is thus likely that any alterations in basal or maximal myocardial blood flow, contributing to an abnormal coronary vasodilator reserve, within the first week were missed. Nevertheless, these data are consistent with our study since we have shown that the coronary vasodilator reserve returns to normal between 7 days and 3 months. Using thallium-201 stress imaging, this delay in the recovery of perfusion has also been reported in patients persisting between 4 and 18 days after PTCA (Manyari et al, 1988).

A reduction in "perfusion defects" within 72 hours of angioplasty implying an early recovery of myocardial perfusion has been reported using PET (Nienaber et al, 1991), but this was a qualitative analysis with no quantification of myocardial blood flow or measurement of the vasodilator reserve, and hence it does not define the

vasodilator abnormalities present in the angioplasty region relative to a remote region. Studies by Lassar et al (Lassar et al, 1984) and Hodgson et al (Hodgson et al, 1987) both reported that the coronary blood flow reserve measured in dilated vessels immediately after angioplasty was similar to that measured in normal coronary arteries. However, these studies less reliable since the xenon-133 techniques tend to overestimate coronary flow, and because the stimulus for coronary vasodilatation (iodinated contrast medium or small doses of papaverine) may have been variable.

Mechanisms for the Alteration in Resistive Vessel Function after Angioplasty

Several vascular beds including the coronary circulation autoregulate arteriolar resistance according to perfusion pressure (Rouleau et al, 1979). The effects of a prolonged reduction in perfusion pressure (resulting from an epicardial coronary artery stenosis) on resistive vessel autoregulation are not well documented. It is possible that prolonged resistive vessel dilatation resulting from a low perfusion pressure distal to the stenosis might cause a transient inability of these vessels to autoregulate in response to a sudden restoration of a normal perfusion pressure (Fischell et al, 1990). If, after the abrupt restoration of a normal coronary perfusion pressure by successful PTCA, the resistive vessels failed to vasoconstrict, then an increase in basal myocardial blood flow and a fall in the maximal myocardial blood flow/basal myocardial blood flow ratio, ie. the coronary vasodilator reserve, would be expected, as was observed in this study.

Alternatively, basal myocardial blood flow may have been elevated due to the release of vasoactive agents at the time of angioplasty, eg. from platelets activated by and adherent to the site of dilatation, the injured vessel wall, or even the myocardial cells themselves, perhaps as a result of transient ischaemia. This is a less likely explanation since the increase in basal myocardial blood flow persisted for at least 7

days and factors directly related to the procedure would be expected to resolve by that time. It is more probable that resistive vessel function is abnormal before angioplasty due to the altered autoregulatory mechanisms to compensate for the stenosis as discussed above.

The maximal myocardial blood flow response to dipyridamole was also reduced immediately after angioplasty. The mechanism underlying this response is uncertain and several can be considered. Vasoconstrictor substances released by platelets at the angioplasty site may limit maximal vasodilatation (McFadden et al, 1991). Embolisation of the distal coronary circulation with platelet aggregates or fragments of thrombus might result in transient alterations in coronary blood flow which later regress (Falk, 1985). One animal study has shown that microembolisation of the coronary circulation increased resting coronary blood flow and decreased the maximal hyperaemic coronary blood flow response (Hori et al, 1986). Other factors related to the presence of a severe stenosis such as recurrent ischaemia before and during angioplasty might have produced transient changes in the maximal coronary vasodilator response. Although it has been suggested that short term administration of calcium channel blockers can reduce vasodilator capacity (Dymek & Bache, 1984), which were avoided in our study, Wilson et al found no difference in the coronary vasodilator reserve in patients being treated with nitrates, β-blockers and calcium channel blockers compared with those off anti-anginal medication (Wilson et al, 1988).

Potential Limitations

Intravenous dipyridamole was selected as the vasodilator of choice so that data derived from Doppler catheterisation could be compared with PET at an equivalent vasodilator stress. Although the vasodilator response may vary amongst individuals leading to a degree of scatter in the absolute value of vasodilator blood flow, the

advantage of positron emission tomography is that all regions of of left ventricular myocardium may be studied simultaneously thus allowing each patient to act as his own control.

The problem of whether maximal vasodilatation has occurred or not still remains however. The standard dose of dipyridamole given intravenously has been 0.56 $mg \cdot kg^{-1}$ over 4 minutes in previous studies (Chapter 2, Section 2.3.2.1), but there is no pharmacological rationale for this arbitrary dose, it having been derived originally by extrapolation from a total dose used. Although one may question whether or not maximal vasodilatation occurred in all patients, the fact remains that in relative terms, myocardial blood flow and the coronary vasodilator reserve was shown to be significantly different when comparing two regions at the same time in the same individual, and thus in the study population.

Clinical Implications

Two important clinical implications arise out of this study. i) A satisfactory angiographic result does not indicate the immediate return of the behaviour of the coronary circulation to normal. However, in the absence of restenosis, basal myocardial blood flow, the myocardial blood flow response to dipyridamole, and thus, the coronary vasodilator reserve will return to normal over a variable period, of at least one week. This observation may account for the finding of a positive exercise test several days after angioplasty in the absence of restenosis (El-Tamimi et al, 1990). Thus, exercise tests and other non-invasive stress tests will be more reliable in detecting restenosis if performed at least one week after angioplasty. Conversely, measurement of the coronary vasodilator reserve after angioplasty cannot be used to assess the adequacy of the procedure since it may take several days before it has normalised. ii) Resistive vessel function may be altered in patients with coronary artery disease. Immediately after removal of the stenosis by angioplasty, there is no

improvement in coronary vasodilator reserve suggesting that resistive vessel dysfunction in patients with coronary artery disease may contribute to the development of myocardial ischaemia.

Conclusions

After successful angioplasty, basal myocardial blood flow is increased for at least 7 days in the myocardial region subtended by the previously stenosed artery. The dipyridamole-induced increase in maximal myocardial blood flow is also impaired for at least 24 hours after the procedure. The coronary vasodilator reserve in the angioplasty region is therefore impaired for at least 7 days after angioplasty. Thus, there is abnormal resistive vessel function in the coronary vascular bed distal to a coronary artery stenosis which persists for between 7 days and 3 months following the procedure.

CHAPTER 6. NITRIC OXIDE PRODUCTION AND ALTERED CORONARY RESISTIVE VESSEL FUNCTION AFTER CORONARY ANGIOPLASTY

6.1 Abstract

The coronary vasodilator response to dipyridamole is impaired immediately after successful coronary angioplasty due to an alteration in resistive vessel dilatation. Impaired production or release of nitric oxide in the resistive vessels may account for this alteration. As the vasodilator response to exogenous nitrates is enhanced by the removal of endothelium and by inhibition of nitric oxide synthesis, after angioplasty we have infused large doses of sodium nitroprusside, a nitric oxide donor, following dipyridamole to test this hypothesis. The coronary vasodilator reserve (maximal/basal coronary blood flow) to intravenous dipyridamole was measured by Doppler catheterisation after angioplasty in 10 male patients (mean age 53±10 years) with single vessel coronary artery disease. At the peak effect of dipyridamole, incremental doses of sodium nitroprusside (4-50 $\mu g \cdot min^{-1}$) were given intracoronary until systolic blood pressure fell by ≥5 mmHg. After successful angioplasty, mean coronary blood flow increased from 32.4±13.2 to 53.4±23.3 $ml \cdot min^{-1}$ at the peak dipyridamole effect ($p<0.05$ vs. basal), giving a coronary vasodilator reserve of 1.77±0.64. At the dose of nitroprusside before a fall in systolic and mean arterial pressure, mean coronary blood flow was 49.5±20.4 $ml \cdot min^{-1}$ (p=NS vs. dipyridamole). Immediately after the systolic pressure fall, mean coronary blood flow was 52.2±18.0 $ml \cdot min^{-1}$ (p=NS vs. peak dipyridamole, and nitroprusside before systolic pressure fall).

Immediately after successful angioplasty, the maximal coronary blood flow velocity increase to dipyridamole was markedly impaired. Intracoronary sodium nitroprusside in doses sufficient to cause ultimately a fall in blood pressure did not

augment the dipyridamole-induced increase in coronary blood flow velocity. Thus, reduced production or release of nitric oxide in the coronary circulation does not seem to be responsible for the impaired coronary vasodilator reserve after angioplasty.

6.2 Introduction

A large number of patients have a positive exercise test after successful coronary angioplasty. In one study, 41% of patients had a positive exercise test after 6 months despite a mean residual stenosis of only 54% (Parisi et al, 1992) and other studies have reported similar findings after angioplasty (Massa et al, 1989; Schroeder et al, 1989). Since such a stenosis would not be expected to significantly reduce the coronary vasodilator reserve in its own right (Gould et al, 1974), it is likely that this occurs because of resistive vessel dysfunction (Pupita et al, 1990). Intracoronary Doppler flow measurements in the stenosis-related artery after angioplasty from several groups have shown a reduced response to intravenous dipyridamole (Kern et al, 1989; Zijlstra et al, 1988; Uren et al, 1993a) and to intracoronary papaverine (Wilson et al, 1988). Furthermore, as we demonstrated in chapter 5, positron emission tomography has confirmed that the reduced vasodilator response to dipyridamole in myocardium subtended by the angioplasty vessel persists for up to 24 hours after the procedure (Uren et al, 1993a).

The mechanisms leading to this reduced coronary vasodilator reserve in the vascular territory subtended by a successfully dilated coronary artery are not well understood. Endothelial dysfunction and denudation at the lesion site due to balloon dilatation which leads to a loss of control of vasomotor tone has been implicated in early stenosis vasoconstriction and even abrupt closure following angioplasty (Lazzam et al, 1992). A defective production or release of endothelium-derived relaxing factor (EDRF, which may be identical to nitric oxide (Palmer et al, 1987) leading to an

enhanced vasomotor tone in coronary resistive vessels has been proposed as a possible explanation for the reduced vasodilator response in patients with coronary artery disease (Kuo et al, 1992). It has been demonstrated in vitro that vasodilatation to exogenous nitrates is enhanced by defective EDRF production either by endothelium removal (Shirasaki & Su, 1985), or by nitric oxide synthesis inhibition (Shirasaki & Su, 1985; Moncada et al, 1991), which appears to be related to an up-regulation of soluble guanylate cyclase (Moncada et al, 1991). If the impairment of the vasodilator response after angioplasty was due to reduced production of nitric oxide, then there would be an increase in the vasodilator response with delivery of a high concentration of nitric oxide to the coronary microcirculation. In order to test this hypothesis, we infused intracoronary high doses of sodium nitroprusside, a nitric oxide donor (Feelisch et al, 1987), after intravenous dipyridamole, into the index vessel after successful angioplasty. In some of the patients, we also performed the same study before angioplasty in order to assess the response of the coronary vascular bed before the procedure.

6.3 Methods

6.3.1. Patient Population

Ten male patients (mean age 53±10 years, range 40-72) with chronic stable angina were studied. All had a positive exercise test and normal left ventricular function, and patients who had suffered a previous myocardial infarction or unstable angina pectoris were excluded. All had proximal left anterior descending coronary artery disease and were undergoing routine coronary angioplasty. The left circumflex and right coronary arteries in these patients were angiographically normal.

6.3.2. Study Protocol/

6.3.2. Study Protocol

The protocol was approved by the Research Ethics Committee of our institution and all patients gave informed and written consent.

All anti-anginal medication (except sublingual nitroglycerin) was discontinued at least 48 hours prior to cardiac catheterisation and angioplasty. No patient took nitroglycerin within 12 hours of the study. All patients were taking aspirin 300 mg daily. Intravenous heparin (10,000 units) was given immediately prior to the study. Angioplasty was performed as clinically indicated. Coronary blood flow velocity and coronary vasodilator reserve (defined as post-dipyridamole coronary flow/basal coronary flow) was measured in 5 patients before, and in all 10 patients after angioplasty as described below.

6.3.2.1 Cardiac Catheterisation and Doppler Flow Velocity Measurement

Coronary angiography was performed as clinically indicated in order to best demonstrate the lesion morphology. On completion, an 8-French gauge guiding catheter (Cordis Ltd., Brentford, Middlesex) was inserted into the left coronary ostium. A 0.010 or 0.012 in. "Hi-Torque" guide wire (Advanced Cardiovascular Systems Inc., Santa Clara, Calif., USA) was advanced across the coronary lesion. Prior to angioplasty, in 5 of the patients in whom there was no evidence of myocardial ischaemia (chest pain or electrocardiogram changes), a 3-French gauge Doppler flow catheter (Model No. DC 201; Millar Instruments Inc. Houston, Texas, USA) with a 20 MHz pulsed Doppler crystal, referenced to zero and calibrated, was advanced over the guide wire. The tip of the Doppler catheter was positioned up to 5 mm proximal to the stenosis (Wilson et al, 1985). The Doppler velocimeter range control was adjusted to obtain an optimal audio signal and both phasic and mean tracing of maximal resting coronary blood flow velocity.

Heart rate, systemic arterial pressure (right iliac artery) and coronary blood flow velocity were measured continuously throughout the procedure. Coronary resistive vessel dilatation was induced with intravenous dipyridamole 0.5 $mg \cdot kg^{-1}$ over 4 minutes. At the peak mean coronary flow velocity following the infusion of dipyridamole, an intracoronary infusion of sodium nitroprusside was given in incremental doses of 4, 8, 16, 32 and 50 $\mu g \cdot min^{-1}$ each for 1 minute. The sodium nitroprusside infusion was stopped as soon as systolic blood pressure fell by ≥5 mmHg. Coronary arteriograms were obtained under basal conditions, at the peak coronary blood flow velocity following dipyridamole, and during sodium nitroprusside infusion immediately following a ≥5 mmHg drop in systolic blood pressure. Angioplasty was then performed entirely as clinically indicated (ACS RX balloon angioplasty catheter; Advanced Cardiovascular Systems Inc., Santa Clara, Calif., USA). Following successful angioplasty, the balloon catheter was removed leaving the guide wire in situ. The Doppler flow catheter was positioned as described above in all 10 patients. Measurement of coronary blood flow velocity after intravenous dipyridamole and during intracoronary nitroprusside were obtained as described above, together with coronary arteriography at the same time intervals. Patients in whom it was necessary to give nitrates during the angioplasty were excluded from further study.

6.3.2.2. Quantitative Coronary Arteriography

Coronary arteriograms were analyzed by an automated edge contour detection computer analysis system (Cardiovascular Angiographic Analysis System [CAAS], Pie Medical Equipment B.V., Maastricht, The Netherlands) (Reiber et al, 1984). This method which involves detection of the boundaries of the relevant coronary artery segment from the optically magnified and video digitalised region of interest is described in Chapter 5 (Section 5.3.2.2). In this study, stenosis severity was also

expressed as percentage reduction of the internal lumen diameter relative to the angiographically normal proximal coronary segment, the reference segment.

6.3.2.3. Data Analysis

Heart rate, coronary flow velocity, and systemic blood pressure were measured continuously during the study. Data were analysed at i) basal, ii) the peak effect of dipyridamole, iii) the sodium nitroprusside dose before a reduction in systolic pressure, and iv) the sodium nitroprusside dose after a ≥5 mmHg reduction in systolic pressure. Mean coronary blood flow was calculated from the product of the coronary flow velocity ($cm \cdot s^{-1}$) and the cross-sectional area of artery at the tip of the Doppler catheter (cm^2) and expressed in $ml \cdot min^{-1}$. Coronary resistance was calculated as the quotient of mean arterial pressure to mean coronary blood flow and expressed in $mmHg \cdot min \cdot ml^{-1}$.

6.3.3. Statistical Analysis

All data are expressed as mean±SD. Student's t test was used to compare any pair of mean group values. Simultaneous comparison of more than two mean values was performed using one-way analysis of variance (ANOVA), and Fisher's least significant difference method was subsequently applied to localize the source of the difference. A p value <0.05 was considered statistically significant.

6.4 Results

6.4.1. Quantitative Coronary Arteriography

(Table 6.1)

The results of quantitative angiography are shown in Table 6.1. In all 10 patients, diameter at the site of the stenosis was increased from 0.62±0.30 to 1.92±0.50 mm

($p<0.0001$) by angioplasty. The reference artery segment was 2.33±0.39 and 2.55±0.53 mm before and after angioplasty (p=NS), respectively. Thus, there was a reduction in percentage lumen stenosis severity from 74.8±10.4 to 25.4±7.6% ($p<0.0001$).

All 10 patients were studied after angioplasty. Dipyridamole had no effect on residual stenosis diameter, although sodium nitroprusside did cause mild dilatation. Dipyridamole did not change significantly the reference artery diameter, although sodium nitroprusside dilated the reference segment. In the 5 patients studied before angioplasty, changes in coronary calibre were not statistically significant (Table 6.1).

6.4.2. Doppler Flow Velocity Measurement After Angioplasty

(Table 6.2 and Figures 6.1 and 6.2)

All 10 patients underwent Doppler catheterisation after angioplasty (Table 6.2). Heart rate increased at the peak effect of dipyridamole, with no significant reduction in systolic, diastolic, or mean arterial pressure. There was an significant increase in mean coronary blood flow after dipyridamole, from 32.4±13.2 to 53.4±23.3 $ml \cdot min^{-1}$ ($p<0.05$ vs. basal) (Figure 6.1), with a reduction in coronary resistance, from 3.95±2.54 to 2.20±1.10 $mmHg \cdot min \cdot ml^{-1}$ ($p<0.05$ vs. basal) (Figure 6.2). The coronary vasodilator reserve was 1.77±0.67. Sodium nitroprusside, at a dose just before a fall in blood pressure, did not change significantly the mean coronary blood flow, 49.5±20.4 $ml \cdot min^{-1}$, or coronary resistance, 2.19±1.14 $mmHg \cdot min \cdot ml^{-1}$ (both p=NS vs. dipyridamole) (Figures 6.1 and 6.2). At the peak systemic effect of sodium nitroprusside, there was a fall in systolic, diastolic and mean arterial blood pressure, but there was no further change in mean coronary blood flow, 52.2±18.0 $ml \cdot min^{-1}$, or coronary resistance, 1.90±0.88 $mmHg \cdot min \cdot ml^{-1}$ (both p=NS vs. dipyridamole) (Figures 6.1 and 6.2). The coronary vasodilator reserve before and after the reduction

Table 6.1. Quantitative Coronary Arteriography

	Basal State	Peak Dipyridamole Effect	Peak Sodium Nitroprusside Effect
Before angioplasty	(n=10)	(n=5)	(n=5)
Stenosis (mm)	0.62±0.30	0.74±0.32	0.73±0.35
Reference (mm)	2.33±0.39	2.28±0.47	2.48±0.55
% stenosis	74.8±10.4%	68.2±10.3%	71.0±10.0%
After angioplasty	(n=10)	(n=10)	(n=10)
Stenosis (mm)	1.92±0.50	1.88±0.49	2.02±0.51*
Reference (mm)	2.55±0.53	2.56±0.42	2.72±0.50†
% stenosis	25.4±7.6% ‡	26.6±14.0% ‡	25.8±11.0% ‡

* $p<0.05$ vs. Dipyridamole, † $p<0.05$ vs. Basal; ‡ $p<0.0001$ vs. before angioplasty. at quantitative coronary arteriography before and after coronary angioplasty under basal conditions, at the peak effect of dipyridamole, and at the peak effect sodium nitroprusside.

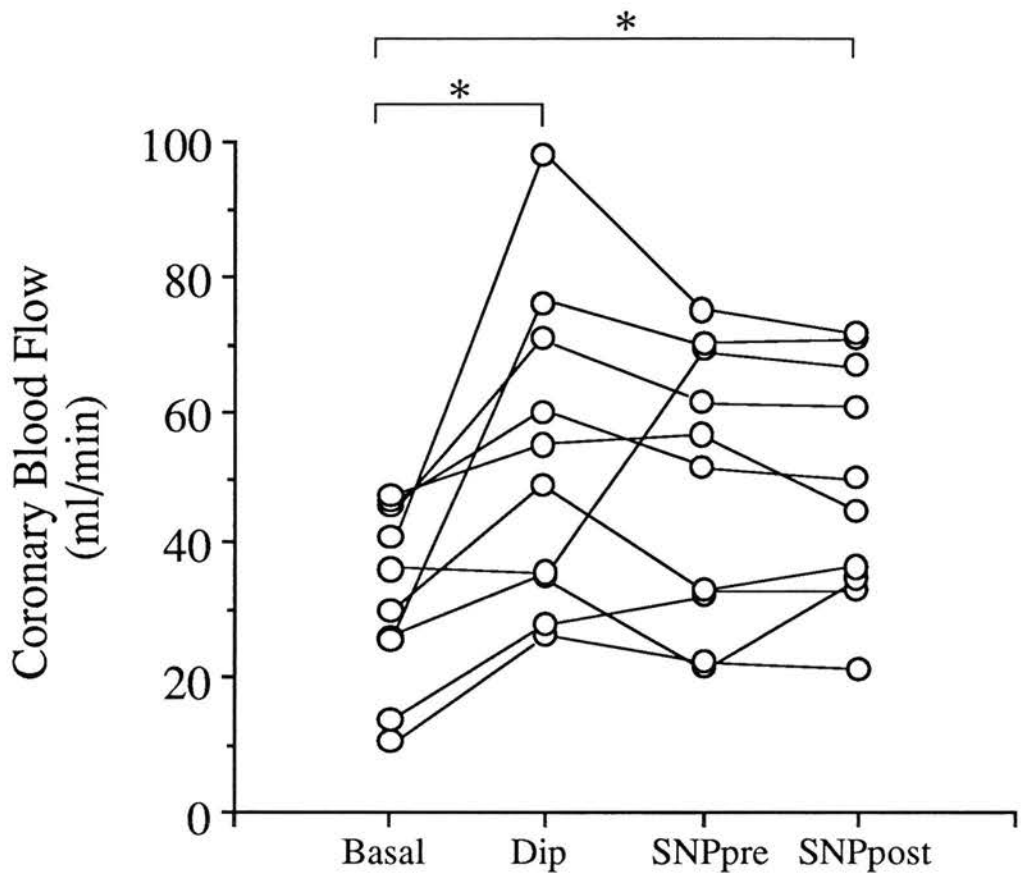


Figure 6.1. Individual coronary blood flows under basal conditions, at peak dipyridamole (Dip), before the sodium nitroprusside-induced fall in systolic blood pressure (SNP_{pre}), and after the sodium nitroprusside-induced fall in systolic blood pressure (SNP_{post}) after angioplasty. * $p<0.05$ vs Basal.

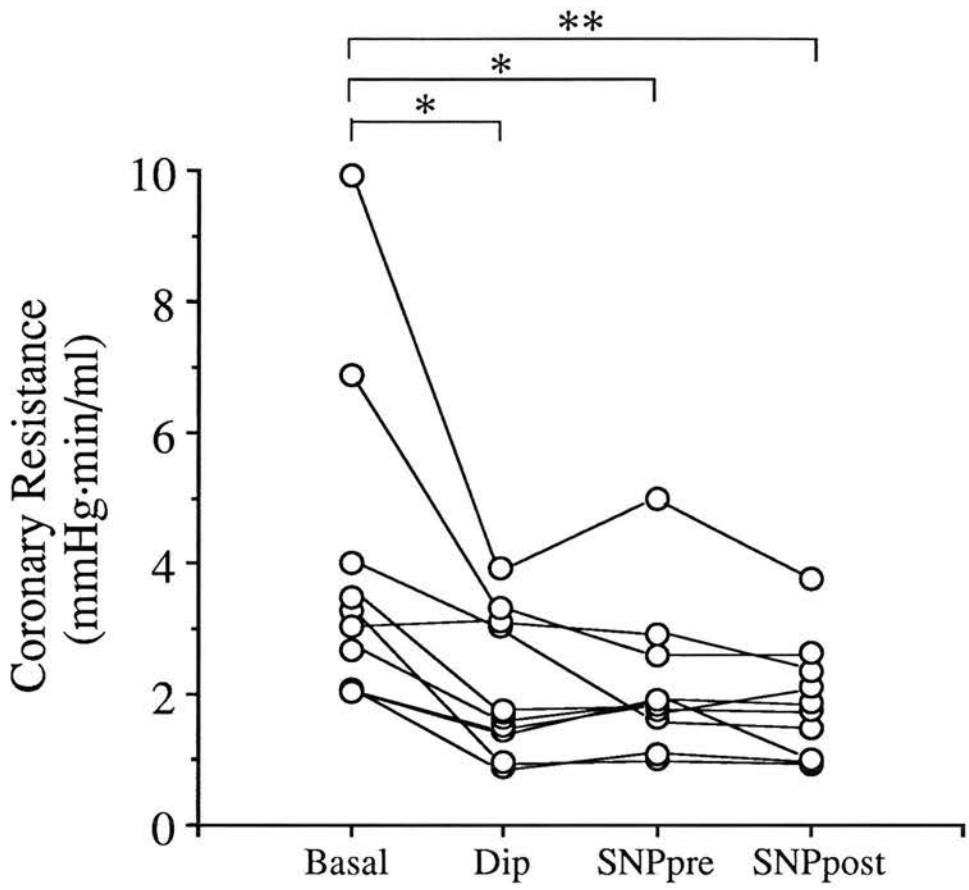


Figure 6.2. Individual coronary resistances under basal conditions, at peak dipyridamole (Dip), before the sodium nitroprusside-induced fall in systolic blood pressure (SNP_{pre}), and after the sodium nitroprusside-induced fall in systolic blood pressure (SNP_{post}) after angioplasty. * $p<0.05$, ** $p<0.01$ vs. Basal.

Table 6.2. Haemodynamic and Coronary Flow Variables in 10 Patients After Angioplasty

	Basal State	Peak Dipyridamole Effect	SNP_{pre}	SNP_{post}
Heart Rate (beats·min^{-1})	68±13	83±18*	81±18*	82±17*
Systolic Blood Pressure (mmHg)	136±22	130±21	134±23	123±20*
Diastolic Blood Pressure (mmHg)	85±13	80±16	73±12	72±14*
Mean Arterial Pressure (mmHg)	101±13	97±17	92±16*	89±15*
Coronary Blood Flow (ml·min^{-1})	32.4±13.2	53.4±23.3*	49.5±20.4*	52.2±18.0*
Coronary Resistance (mmHg·min·ml^{-1})	3.95±2.54	2.20±1.10*	2.19±1.14*	1.90±0.88*
Coronary Vasodilator Reserve	-	1.77±0.67	1.68±0.67	1.78±0.65

* $p<0.05$ vs. Basal. SNP_{pre} = sodium nitroprusside dose before the systolic blood pressure drop, SNP_{post} = sodium nitroprusside dose after the systolic blood pressure drop.

Table 6.3. Haemodynamic and Coronary Flow Variables in 5 Patients Before and After Angioplasty

	Before angioplasty				After angioplasty			
	Basal	Dipyridamole	SNP_{pre}	SNP_{post}	Basal	Dipyridamole	SNP_{pre}	SNP_{post}
Heart rate (min^{-1})	66±9	83±6 †	84±4 †	84±2 †	66±7	79±8 †	78±7 †	80±7 †
Systolic blood pressure (mmHg)	153±20	142±14	149±17	139±18	151±18	141±22	145±23	134±21
Diastolic blood pressure (mmHg)	95±10	87±12	84±11	80±10	91±13	90±15	79±12	79±16
Mean arterial pressure (mmHg)	114±11	107±12	105±11	100±10	109±11	107±15	101±14	96±16
Coronary blood flow ($ml \cdot min^{-1}$)	19.7±6.1	31.1±11.9	37.8±18.6	35.8±19.1	32.9±14.1§	47.7±16.8	50.7±17.0	48.7±14.0
Coronary resistance ($mmHg \cdot min \cdot ml^{-1}$)	6.22±1.77	3.85±0.96 *	3.21±1.15 †	3.28±1.21 †	3.83±1.87 #	2.31±0.88 *§	2.01±0.36 *	1.84±0.6 *§
Coronary vasodilator reserve	-	1.62±0.39	1.86±0.41	1.74±0.43	-	1.53±0.30	1.68±0.70	1.75±0.71

* $p<0.05$, † $p<0.01$ vs. Basal; § $p<0.05$, # $p<0.01$ vs. before angioplasty. SNP_{pre} = sodium nitroprusside dose before the systolic blood pressure drop, SNP_{post} = sodium nitroprusside dose after the systolic blood pressure drop.

of systolic blood pressure with sodium nitroprusside was 1.68±0.67 and 1.78±0.65 (both p=NS vs. dipyridamole).

Comparison of haemodynamic and coronary flow parameters in the 5 patients studied before and after angioplasty. The 5 patients studied before and after angioplasty were compared (Table 6.3). The haemodynamic responses to dipyridamole and sodium nitroprusside were similar before and after angioplasty in the 5 patients. Coronary angioplasty lead to a significant increase in basal mean coronary blood flow, although there was no significant change in the mean coronary blood flow after dipyridamole or during the sodium nitroprusside infusion. Thus, there was no difference in the coronary vasodilator reserve at basal, peak dipyridamole, and during nitroprusside before and after angioplasty. However, after angioplasty, the basal coronary resistance was lower at basal, peak dipyridamole, and during nitroprusside infusion compared to before angioplasty.

6.5 Discussion

This study confirms that the maximal coronary vasodilator response to dipyridamole was markedly impaired immediately after successful coronary angioplasty, with a value similar to that before angioplasty. This impairment is unlikely to be due to a defect in nitric oxide production in the resistive vessels since large doses of sodium nitroprusside, a nitric oxide donor, sufficient to reduce systemic blood pressure, had no further effect on the maximal coronary blood flow response. In the absence of resistive vessel dysfunction, removal of a flow-limiting stenosis would be expected to restore normal maximal coronary blood flow (Wilson et al, 1988). As the residual stenosis severity was around 25% in this study, it is unlikely that the residual epicardial lesion had a flow-limiting effect on the coronary vasodilator

reserve (Gould et al, 1974). Our study thus indicates that there was a clear failure of the resistive vessels to dilate in response to dipyridamole after angioplasty. Furthermore, sodium nitroprusside did not normalise the coronary blood flow response to dipyridamole (although it did have a mild vasodilating effect both at the site of the stenosis and on the segment of epicardial coronary artery proximal to the stenosis site, indicative of a local action in the coronary circulation). Thus, reduced production or activity of nitric oxide in the coronary circulation does not seem to be responsible for the impaired vasodilator reserve after coronary angioplasty.

Nitric Oxide and Resistive Vessel Function in Atherosclerosis

There is evidence to suggest that the atherosclerotic process in large epicardial coronary arteries impairs their ability to dilate in response to increasing blood flow, and that this may be due to endothelial cell dysfunction (Cox et al, 1989). This loss of endothelial responsiveness to blood flow and also to acetylcholine occurs before the development of flow-limiting epicardial coronary artery stenoses (McLenachan et al, 1991). In the coronary resistive vessels, although overt atherosclerosis does not develop, endothelial cell dysfunction may occur (Kuo et al, 1992). In an experimental primate model, Sellke et al showed that impaired resistive vessel dilatation to endothelial-dependent vasodilators and constriction to acetylcholine occurs, although sodium nitroprusside induced vasodilatation of a similar magnitude in the microvessels from both atherosclerotic and control animals (Sellke et al, 1990). Furthermore, in man, Zeiher et al reported that impaired resistive vessel dilatation to acetylcholine occurs in myocardium subtended by an angiographically normal coronary artery in patients with disease in other arteries, despite a normal vasodilator response to papaverine (Zeiher et al, 1991). These studies indicate a preserved ability of vascular smooth muscle in resistive vessels to vasodilate, despite endothelial cell dysfunction. The alteration of resistive vessel function in response to endothelium-dependent

vasodilatation in these circumstances may involve a deficiency in the production or release of nitric oxide, as it is possible to restore maximal endothelium-dependent vasodilatation in resistive vessels from atherosclerotic animals by incubation with L-arginine (Kuo et al, 1992), the substrate for nitric oxide synthesis (Palmer et al, 1988). Again, in this study, the vasodilator response to sodium nitroprusside was similar on comparing either endothelium-denuded or atherosclerotic vessels with normal controls, implying preserved vascular smooth muscle responsiveness (Kuo et al, 1992).

Abnormal Resistive Vessel Function After Angioplasty

Whether the process leading to impaired resistive vessel vasodilatation arises acutely as a direct consequence of the angioplasty procedure itself, or occurs as a chronic adaptation to a flow-limiting epicardial stenosis, is unknown. Brief reversible myocardial ischaemia in an animal model can lead to a persistent impairment of vasodilator responsiveness (for at least 4 hours), independent of the degree of reduced myocardial contractility - so-called "microvascular stunning" (Nicklas & Gips, 1989; Bolli et al, 1990). Vasoconstrictor substances, such as serotonin released by platelets at the angioplasty site, cause diffuse constriction of the distal coronary arteries limiting maximal vasodilatation (McFadden et al, 1991). Additionally, embolisation of the distal coronary circulation with platelet aggregates or clot debris may both increase resting coronary blood flow and decrease the maximal hyperaemic coronary blood flow response (Hori et al, 1986).

The effect of chronic reduction in perfusion pressure (resulting from the epicardial coronary artery stenosis) on resistive vessel autoregulation is another possible cause of altered vasoreactivity. Vasoconstriction occurs in epicardial coronary arteries distal to a stenosis after successful angioplasty, and is proportional to the severity of the previous stenosis (Fischell et al, 1990). Coronary resistive vessels may also adapt structurally to a persistently low perfusion pressure, and one animal study has shown

that after several weeks a proximal epicardial coronary stenosis causes a reduction in the ratio of wall area to lumen diameter in the microvessels, associated with changes in membrane proteins on histochemical analysis (Mills et al, 1991).

Our study indicates that reduced nitric oxide production or release at the level of the coronary resistive vessels is not the cause of the impaired coronary vasodilator reserve after angioplasty. There is now evidence that other non-nitric oxide dependent endothelium-derived relaxing factors exist, and that these play a major role in the vasodilator responsiveness of the coronary microcirculation (Vanhoutte & Shimokawa, 1991). Thus, the impairment in the coronary vasodilator reserve may occur as a result of an alteration in production of such factors in the coronary microcirculation and our data do not exclude such hypotheses.

Conclusions

Immediately after successful angioplasty, the maximal mean coronary blood flow increase to dipyridamole was markedly impaired, with a coronary vasodilator reserve similar to that seen before angioplasty. Administration of intracoronary sodium nitroprusside in doses sufficient to cause mild dilatation of epicardial coronary arteries and a fall in systemic blood pressure did not augment the dipyridamole-induced increase in mean coronary blood flow after angioplasty. Thus, reduced nitric oxide production or release in the coronary circulation is not the cause of the impaired coronary vasodilator reserve seen after coronary angioplasty.

CHAPTER 7. ALTERED CORONARY RESISTIVE VESSEL FUNCTION AND REGIONAL METABOLISM IN MYOCARDIUM SUBTENDED BY NORMAL ARTERIES IN CORONARY ARTERY DISEASE

7.1 Abstract

There is evidence that following a myocardial infarction, structural and functional changes occur in the remote myocardium which is often subtended by non-diseased vessels. However, it is not known whether any changes occur in regions subtended by angiographically normal arteries remote from a zone of ischaemic, but non-infarcted myocardium in humans. The objective of this study was to investigate coronary vasodilator reserve and myocardial metabolism in regions subtended by angiographically normal arteries in patients with stable angina pectoris and coronary disease elsewhere. Regional coronary vasodilator reserve was measured using positron emission tomography in 12 patients with single vessel disease and 13 controls. In a second group of 10 patients and 6 controls, simultaneous arterial and great cardiac vein catheterisation was done at rest and during atrial pacing to measure myocardial metabolism in regions subtended by a diseased artery (left anterior descending artery) or by an angiographically normal artery (left anterior descending artery in patients with right or circumflex disease). Coronary vasodilator reserve was 1.80±0.24 in regions subtended by a stenosis and 2.73±0.25 in remote regions ($p<0.01$ vs. stenosis region). Both values were lower than that in the controls (4.07±0.21; $p<0.01$). In the second group, no differences in myocardial metabolism were present at rest. During pacing, there was net lactate production in the diseased region, -18± 27% ($p<0.05$ vs. both remote and control), compared to net extraction in

the remote region, 38±17%, and control, 26±11%. Glucose and alanine extraction were increased both in the diseased region (8±6% and 6±6%), and in the remote region (6±3% and 4±3%), compared to control (2±3% and -1± 3%, $p<0.05$ vs. both diseased and remote).

In conclusion, in patients with stable angina and single vessel disease, the coronary vasodilator reserve is reduced in regions subtended by angiographically normal arteries. In addition, such regions have different glucose and alanine handling during pacing in the absence of lactate production.

7.2 Introduction

There is increasing evidence that following a myocardial infarction, structural and functional changes occur in the remote non-infarcted regions of the left ventricle, which are often subtended by non-diseased vessels, a process termed ventricular remodelling. Non-infarcted myocardium is subjected to increased wall stress to compensate for the loss of contractile tissue. This leads to lengthening and hypertrophy of the non-infarcted myocytes which, with time, may progress to dilatation and failure of the left ventricle (Pfeffer & Braunwald, 1990). Transient myocardial ischaemia may also lead to an increase in left ventricular dimensions associated with regional hyperfunction in remote non-ischaemic areas, both in animals (Naccarella et al, 1984; Smalling et al, 1986; Buda et al 1990), and in man (Jaarsma et al, 1986; Serruys et al, 1986). However, it is not known whether patients with recurrent myocardial ischaemia, but without evidence of previous infarction, may develop alterations of cardiac function in the remote regions subtended by angiographically normal coronary arteries.

The aim of this study was to assess whether alterations of myocardial blood flow and metabolism can be demonstrated in areas of myocardium subtended by

angiographically normal arteries, in patients with chronic stable angina and coronary artery disease elsewhere.

7.3 Methods

7.3.1. Patient Population

Twenty two patients with coronary artery disease and normal left ventricular function were studied. Coronary and left ventricular angiograms were analysed by an automated edge contour detection computer analysis system to derive coronary artery stenosis severity and global and regional left ventricular function. None of the patients had a clinical history or ECG evidence of previous myocardial infarction. There was no evidence of valvular or primary cardiac disease in any of the patients. In addition, there was no history of diabetes mellitus or systemic hypertension in any patient.

In 12 patients (Group 1), regional myocardial blood flow and coronary vasodilator reserve were assessed by positron emission tomography. In the other 10 patients (Group 2), myocardial metabolism was measured by simultaneous arterial and great cardiac vein catheterisation at rest and during pacing stress.

i) Group 1

(Table 7.1)

Twelve patients (10 male, mean age 60 years, range 47-76) with significant (>70% diameter stenosis) single vessel coronary artery disease were studied. The remaining coronary arteries in these patients were angiographically normal (Table 7.1). All patients had chronic stable angina pectoris and ≥ 0.1 mV ST segment depression on the ECG during exercise. Data from these patients were compared with a group of 13 normal male subjects (mean age 32 years, range 21-43).

Table 7.1. Coronary Arteriography in Group 1 Patients

Patient	Age (yrs)/ gender	Extent of Coronary Artery Disease (% diameter stenosis and site)			PET Remote Region
		Left Anterior Descending Artery	Left Circumflex Artery	Right Coronary Artery	
1	47/F	80% proximal	ANA	ANA	Inferior
2	52/M	occlusion	ANA	ANA	Inferior
3	56/F	77% proximal	ANA	ANA	Inferior
4	61/M	occlusion	ANA	ANA	Inferior
5	61/M	85% proximal	ANA	ANA	Inferior
6	64/M	occlusion	ANA	ANA	Inferior
7	65/M	occlusion	ANA	ANA	Inferior
8	49/M	ANA	88% proximal	ANA, non-dominant	Anterior
9	54/M	ANA	ANA	occlusion	Anterior
10	63/M	ANA	73% proximal OM_1	ANA, non-dominant	Anterior
11	70/M	ANA	ANA	81% proximal	Anterior
12	76/M	ANA	ANA	82% proximal	Anterior

ANA = angiographically normal artery, OM_1 = first obtuse marginal branch of the left circumflex artery.

Figure 7.1. Coronary venous angiogram (lateral view) obtained following a selective injection of contrast into the left anterior descending coronary artery, which illustrates the course of the great cardiac vein (GCV). The GCV arises in the anterior myocardial wall and runs posteriorly and cranially in the interventricular groove, and then in the atrio-ventricular groove before draining into the coronary sinus which in turn drains into the right atrium.

ii) Group 2

Ten additional patients underwent simultaneous arterial and great cardiac vein (GCV) catheterisation (Figure 7.1) at rest and during maximal atrial pacing, following a routine angiographic study. Five patients (Group 2a: mean age 54 years, range 46-65) had significant left anterior descending artery (LAD) disease, and 5 patients (Group 2b: mean age 52 years, range 42-62) had significant right coronary (RCA) and/or left circumflex (LCX) artery disease. All patients in group 2b had an angiographically normal LAD (Table 7.2). Since the GCV generally drains blood from the distribution territory of the LAD, measurements in patients with significant disease of the LAD should reflect metabolism in a potentially ischaemic territory (Figure 7.2, panels A and B). In contrast, in patients with significant RCA/LCX disease and a normal LAD, measurements from the GCV should reflect metabolism in myocardium remote from the ischaemic zone (Figure 7.2, panels A, C, D). Metabolic data in groups 2a and 2b were compared with those from a control group of 6 normal subjects (mean age 47 years, range 39-57), which have been described previously (Camici et al, 1989a).

7.3.2. Study Protocols and Data Analysis

7.3.2.1. Quantitative Coronary Arteriography and Left Ventriculography

Selective right and left coronary arteriography in multiple views was performed with the Judkins technique. Coronary arteriograms were analysed by an automated edge contour detection computer analysis system (Cardiovascular Angiographic Analysis System [CAAS], Pie Medical Equipment BV, Masstricht, The Netherlands) (Reiber et al, 1984) (Chapter 5, Section 5.3.2.2). Stenosis severity was expressed as percentage reduction of the internal luminal diameter relative to the estimated diameter interpolated from the diameters at the proximal and distal boundaries of the stenosis (Reiber et al, 1984). Collaterals were graded according to the method of Rentrop et al

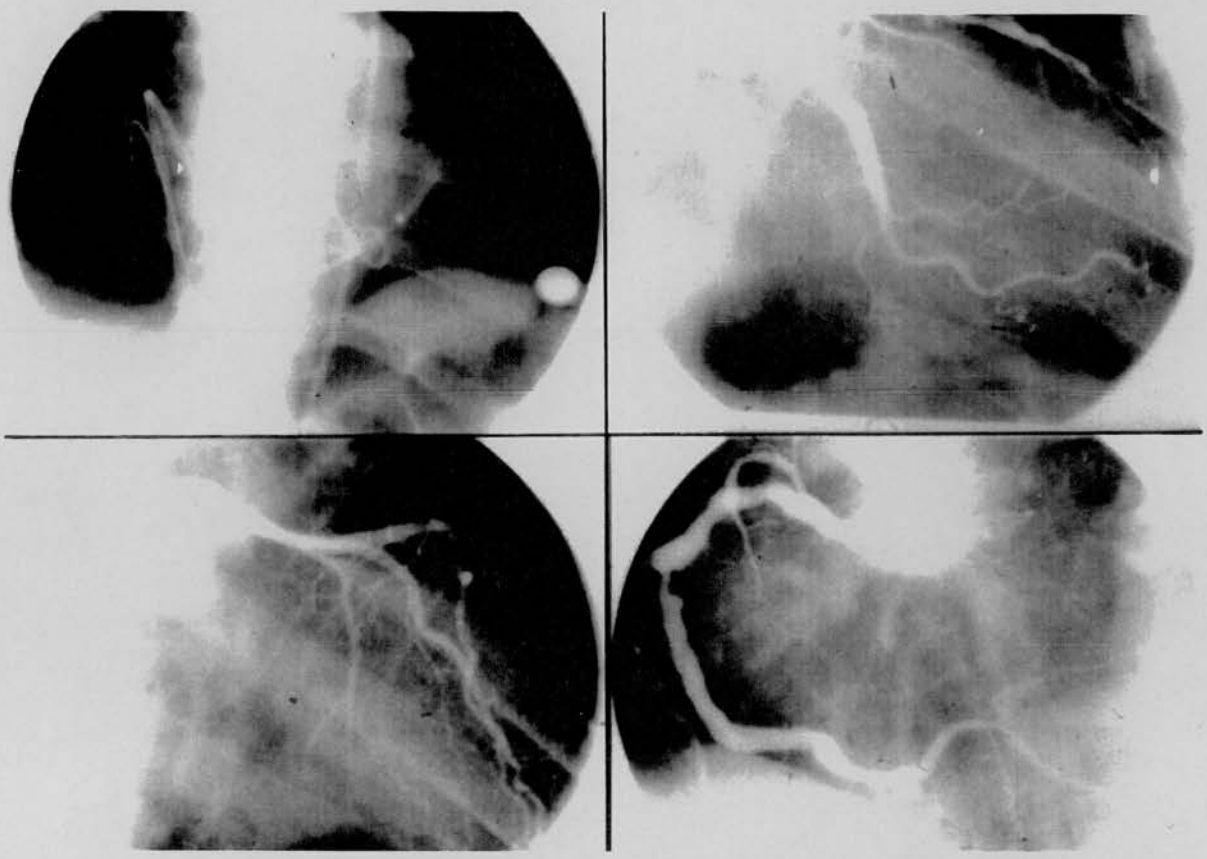


Figure 7.2. Panel A) Antero-posterior view of heart demonstrating a temporary pacing wire in the right atrium, the thermodilution catheter with its tip well advanced into the great cardiac vein, and a pigtail catheter in the left ventricular chamber. **Panel B)** Right anterior oblique view of the left coronary system with a severe proximal stenosis in the left anterior descending artery (group 2a patient). **Panel C)** Right anterior oblique view of an angiographically normal left anterior descending artery (group 2b patient). **Panel D)** Left anterior oblique view of the right coronary artery with a proximal stenosis at the right upper heart border (group 2b patient).

Table 7.2. Coronary Arteriography in Group 2 Patients

Patient	Age (yrs)/ gender	Extent of Coronary Artery Disease (% diameter stenosis and site)			Collateral Grade	GCV Region
		Left Anterior Descending Artery	Left Circumflex Artery	Right Coronary Artery		
Group 2a						
1	46/M	73% proximal at bifurcation with $LADD_1$	min.irreg.	mid-RHB occlusion	Grade 3 from RCA to RCA Grade 3 from LCX to RCA	Ischaemic
2	48/M	>95% proximal; 74% $LADD_1$	OM_1 occlusion	68% distal	Grade 2 from RCA	Ischaemic
3	49/M	>95% proximal	ANA	min. irreg.	Grade 3 from RCA	Ischaemic
4	63/M	>95% proximal	ANA	ANA	Grade 2 from LCX	Ischaemic
5	65/M	71% proximal; >95% $LADD_1$	>95% OM_1	min. irreg.	Grade 1 to OM_1 from RCA	Ischaemic
Group 2b						
1	42/M	ANA	min. irreg.	proximal occlusion	Grade 3 from LCX	Remote
2	49/M	ANA	>95% OM_1	mid-RHB occlusion	Grade 1 from LAD	Remote
3	53/M	ANA	47% mid-vessel stenosis	mid-RHB occlusion	Grade 3 from LAD and LCX	Remote
4	55/M	ANA	43% mid-vessel stenosis	84% proximal	0	Remote
5	62/M	ANA	min. irreg.	distal occlusion	Grade 1 from LAD and LCX	Remote

ANA = angiographically normal artery, LAD = left anterior descending artery; $LADD_1$ = first diagonal branch of LAD, LCX = left circumflex artery, min. irreg. = minimal irregularities; OM_1 = first obtuse marginal branch of LCX, RCA = right coronary artery, RHB = right heart border. For collateral grading (see text).

(Rentrop et al, 1985); where 0=none, 1=filling of side branches without visualisation of the epicardial segment, 2=partial filling of the epicardial segment via collateral channels, and 3=complete filling of the epicardial segment.

Global and regional left ventricular function was measured from the 30° right anterior oblique left ventricular cine-angiogram with an automated hard-wired endocardial contour detector linked to a microcomputer (Serruys et al, 1986). This was done under basal conditions in all patients and at maximal pacing in group 2 patients and controls (in one patient of group 2b, it was not possible to repeat left ventriculography at maximal pacing for technical reasons). End-systolic and end-diastolic contours were drawn to exclude the inferior papillary muscle. Systolic regional wall displacement is determined along a system of 20 coordinates based on the pattern of actual endocardial wall motion in normal individuals (Slager et al, 1979). When normalized for end-diastolic volume, the systolic segmental volume change can be considered as a variable of regional pump function, and thus can express quantitatively, the contribution of a particular segment to global ejection fraction. Regional contribution was described in 5 territories: antero-basal, antero-lateral, apical, inferior, and postero-basal (Serruys et al, 1986). In patients with anterior and/or antero-lateral ischaemia, the postero-basal and inferior regions were defined as remote from the ischaemic region, and in those with inferior ischaemia, the anterobasal and anterolateral regions were considered remote.

7.3.2.2. Positron Emission Tomography

Patients abstained from drinking tea or coffee on the morning of the scan and all had been off anti-anginal medication (except for sublingual nitrates) for at least 72 hours. No nitrates were taken for at least two hours before the study.

All PET scans were performed with an ECAT 931-08/12 camera (CTI Inc., Knoxville, Tennessee, USA). Regional myocardial blood flow (MBF, $ml.min^{-1}.g^{-1}$)

was measured in Group 1 subjects using oxygen-15-labelled water ($H_2{}^{15}O$) as a flow tracer using the previously validated $C^{15}O_2$ inhalation technique (Araujo et al, 1991). Measurements were made at rest and 2 minutes after intravenous administration of dipyridamole (0.56 mg·kg^{-1} over 4 minutes). Systemic blood pressure and heart rate were monitored throughout the study. A 12 lead ECG was recorded every minute during the dipyridamole infusion and for up to 10 minutes after the end of infusion.

In patients with LAD disease, the anterior region was designated as the stenosis-related region and the inferior-posterior region as the remote region of interest. In patients with RCA/LCX disease, the inferior-posterior region was designated as the stenosis-related region and the anterior region as the remote region of interest. MBF was measured under baseline conditions and after an i.v. infusion of dipyridamole (0.56 mg·kg^{-1} over 4 minutes). The coronary vasodilator reserve was defined as the quotient of post-dipyridamole MBF to basal MBF. Since no statistically significant differences among the flow rates in the four major myocardial regions (anterior, septal, lateral and inferior-posterior) could be demonstrated in the control subjects, data from patients of group 1 were compared with the average MBF in the controls.

7.3.2.3. Invasive Measurement of Myocardial Substrate Metabolism

Patients had been off anti-anginal medication (except for sublingual nitrates) for at least 72 hours, and were studied after an overnight fast. Studies were performed following routine coronary arteriography. At least 30 minutes were allowed between coronary angiography and the beginning of the study in order to minimize any possible interference of the contrast medium with myocardial metabolism (Wisneski et al, 1982). An 8-F multi-thermistor thermodilution, pacing catheter was introduced through either the right femoral vein or left antecubital vein and advanced through the coronary sinus into the GCV for sampling and measurement of flow in the distribution

territory of the LAD (Ganz et al, 1971; Pepine et al, 1978). A 7-F pigtail catheter was positioned in the left ventricle. The ECG was continuously monitored.

Subjects performed up to 4 pacing steps of 4 minutes duration each, with heart rate increments of 15 beats·min^{-1} for the first three steps, and up to maximal achievable heart rate (exacerbation of symptoms and/or occurrence of atrio-ventricular conduction disturbances) on the last step (no atropine was used). During maximal pacing, at least two minutes were allowed to achieve a steady condition before the arterial and GCV blood samples were obtained, and pressures and GCV flow measured. Paired arterial and GCV blood samples were drawn under basal conditions (samples taken in duplicate) and at maximal pacing for the assay of glucose, free fatty acids (FFA), lactate, pyruvate, alanine, ß-hydroxybutyrate, glycerol, triglycerides and blood gases as previously reported (Camici et al, 1989a).

Myocardial oxygen consumption (MVO_2) was calculated as the product of GCV flow and the arterial-GCV blood O_2 concentration difference; myocardial carbon dioxide production ($MVCO_2$) was calculated similarly, and the respiratory quotient (RQ) calculated as $MVCO_2/MVO_2$ as previously reported (Camici et al, 1989b). Net rates of carbohydrate and lipid oxidation and myocardial energy expenditure were calculated from gas exchange measurements using standard calorimetric equations (Ferrannini et al, 1988). Thus, net carbohydrate oxidation (μmol·min^{-1}) = $0.57 \cdot MVCO_2 - 0.40 \cdot MVO_2$ and net lipid oxidation (μmol·min^{-1}) = $0.146 \cdot (MVO_2 - MVCO_2)$, assuming protein oxidation in the heart is negligible. Using the caloric equivalents of glucose and fat (respectively 673 and 2,398 cal·mmol^{-1}), myocardial energy expenditure = $0.08 \cdot MVO_2 + 0.034 \cdot MVCO_2$. The fractional extraction (E) of substrates was calculated as follows: $([substrate]_{arterial} - [substrate]_{GCV} \div [substrate]_{arterial}) \times 100$. This fractional extraction is the capacity of the myocardium to extract a substrate from the coronary circulation independent of the arterial concentration of the substrate or the coronary blood flow. Total carbohydrate uptake

was calculated as the algebraic sum of the net exchange of glucose and three-carbon compounds (lactate, pyruvate, and alanine, expressed as glucose equivalents). Coronary resistance was calculated as the quotient of mean arterial pressure to GCV flow. The precise details of the procedures used in this invasive metabolic study have been reported elsewhere (Camici et al, 1989a).

The PET scanning protocol and the protocol for intra-cardiac pacing and coronary sinus catheterisation was approved by the Research Ethics Committee of Hammersmith Hospital . All patients gave informed and written consent.

7.3.3. Statistical Analysis

All data are expressed as mean±SD. Student's t test was used to compare any pair of mean group values. Simultaneous comparison of more than two mean values was performed using one-way analysis of variance (ANOVA) for repeated measures, and Fisher's least significant different method was subsequently applied to localize the source of the difference (Godfrey, 1985). A p value <0.05 was considered statistically significant.

7.4 Results

Results from the PET and catheter studies are presented separately for ischaemic and remote myocardium as defined in Methods.

7.4.1. Ischaemic Regions

i) Regional Myocardial Blood Flow by PET

(Table 7.3; Figures 7.3 and 7.4)

Global left ventricular function under basal conditions was normal in all patients in group 1 (Table 7.3). There was a comparable increase in heart rate from basal to peak

Table 7.3. Global and Regional Left Ventriculography in Group 1 Patients

Patients	Stenosis-related artery	LV ejection fraction (53.3-92.6%)	Regional Wall Motion (% of contribution)				
			Antero-basal (14.4-25.4%)	Antero-lateral (10.1-17.3%)	Apical (2.5-6.3%)	Inferior (10.6-18.2%)	Postero-basal (15.7-25.4%)
1	LAD	74.6	17.3	15.6	4.1	16.2	21.4
2	LAD	81.5	21.3	18.1	4.8	17.8	19.4
3	LAD	82.8	21.9	18.4	3.8	18.0	20.7
4	LAD	88.4	25.4	16.0	4.8	18.3	23.9
5	LAD	84.3	21.7	18.2	4.8	17.9	21.7
6	LAD	81.6	21.9	19.1	6.7	15.6	18.3
7	LAD	82.2	21.6	17.6	4.8	17.3	20.9
8	LCX	64.9	18.1	12.9	4.2	14.2	15.5
9	RCA	74.4	18.6	16.2	5.2	17.8	16.7
10	LCX	80.5	15.7	18.2	6.3	22.8	17.5
11	RCA	73.1	13.5	17.3	7.7	18.4	16.1
12	RCA	79.1	27.0	16.2	2.5	15.6	17.8
Mean±SD		79.0±6.2	20.3±3.8	17.0±1.7	5.0±1.4	17.5±2.1	19.2±2.8

The normal ranges of regional contribution to left ventricular function by the CREF method are in parentheses.
LV = left ventricular; LAD = left anterior descending artery, LCX = left circumflex artery, RCA = right coronary artery.

dipyridamole in patients and controls, (64±10 to 87±14 beats·min^{-1}, and 67±10 to 92±14 beats·min^{-1}, respectively). Similarly, the increase in the rate-pressure product from basal to peak dipyridamole was comparable in patients and controls, (8,550±1830 to 11,760±2250 mmHg·min^{-1}, and 7,640±1630 to 10,480±1660 mmHg·min^{-1}, respectively). Systolic blood pressure at basal and peak dipyridamole was higher in patients compared to controls, (132±19 to 137±18 mmHg, and 115±11 to 115±11 mmHg; $p<0.05$ and $p<0.01$ vs. basal, respectively). In the ischaemic region, resting MBF was 0.96±0.20 ml·min^{-1}·g^{-1}, and increased to 1.73±0.91 ml·min^{-1}·g^{-1} after dipyridamole (Figure 7.3), giving a coronary vasodilator reserve of 1.80±0.82 (Figure 7.4).

ii) Myocardial Metabolism

(Tables 7.4, 7.5, and 7.6)

There was a reduction in left ventricular ejection fraction from basal to maximal pacing, from 81.8±10.0 to 61.9±6.9% ($p<0.01$) in the group 2a patients, and from 72.4±7.6 to 57.2±5.5% ($p<0.05$) in the group 2b patients (Table 7.4), while the ejection fraction was not significantly changed by pacing in the control group from 71.0±7.1 to 66.0±4.9% (p=NS). In group 2a, resting haemodynamic parameters were comparable to those in controls, except for a higher systolic blood pressure (148±17 vs. 124±16 mmHg, $p<0.05$) (Table 7.5). At rest, myocardial substrate utilisation in patients was similar to that in the controls (Table 7.6). All the patients in group 2a developed chest pain, ≥0.1 mV ST segment depression and wall motion abnormalities on left ventriculography at maximal pacing (Table 7.4). This occurred at lower heart rates than in the controls (126±21 vs. 159±23 beats.min^{-1} respectively, $p<0.05$) (Table 7.5). The percent increment in GCV flow during atrial pacing was less in group 2a than in the controls, although this difference did not achieve statistical significance (76±64% vs 133±66%, p=NS). The metabolic data during maximal

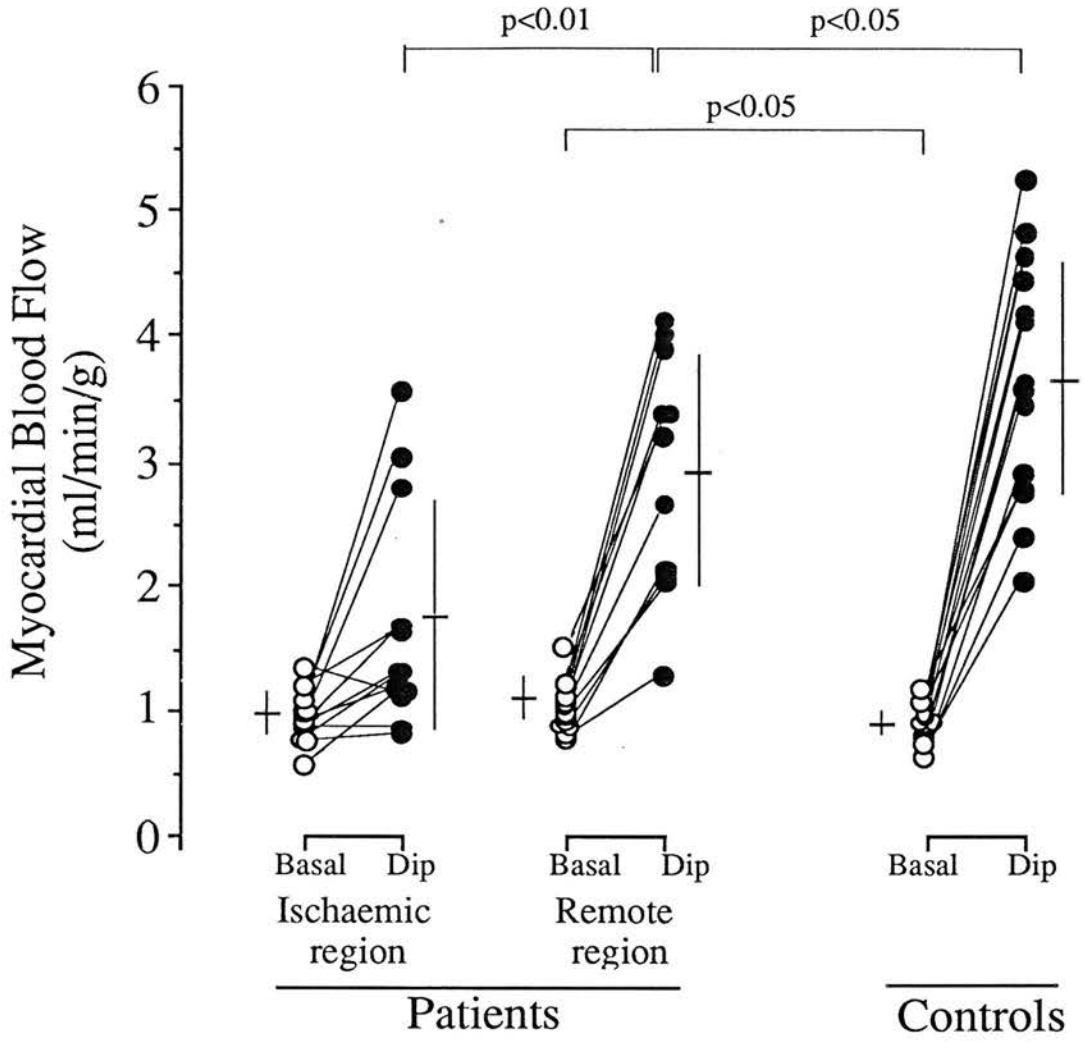


Figure 7.3. The myocardial blood flow at basal (open symbols) and after intravenous dipyridamole (black symbols) in the ischaemic region and remote region of patients with single vessel coronary artery disease (group 1), and in normal control subjects.

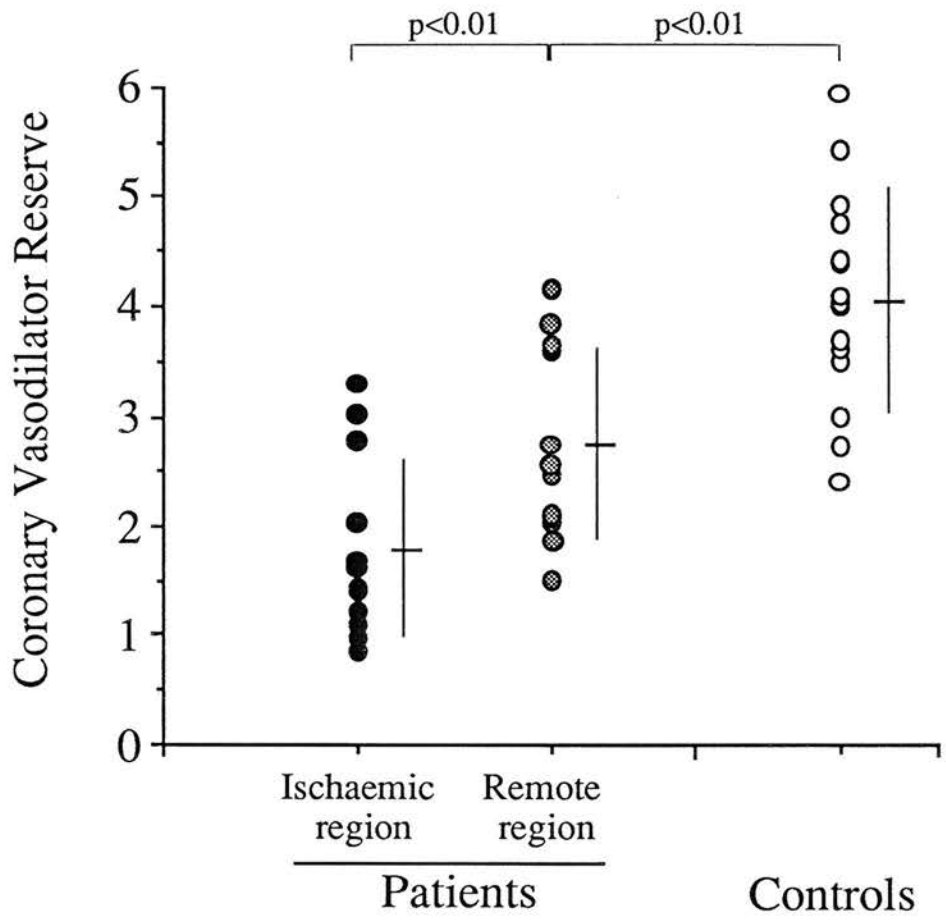


Figure 7.4. The coronary vasodilator reserve (defined as the ratio of post-dipyridamole myocardial blood flow to basal myocardial blood flow) in the ischaemic region and remote region of patients with single vessel coronary artery disease (group 1), and in normal control subjects.

pacing are summarized in Table 7.6. The group 2a patients developed metabolic changes characteristic of myocardial ischaemia, i.e. increased glucose extraction with net lactate release. The respiratory quotient (RQ) at maximal pacing in the ischaemic territory was not different from the respective basal value (0.72±0.03 at basal vs. 0.73±0.18 at pacing), whereas in normal subjects, RQ values increased during pacing (0.72±0.10 at basal vs. 0.84±0.15 at pacing, $p<0.05$) indicating increased oxidation of carbohydrate fuel.

7.4.2. Remote Regions

i) Regional Myocardial Blood Flow by PET

(Figures 7.3 and 7.4)

Resting MBF in the remote region, 1.06±0.19 $ml \cdot min^{-1} \cdot g^{-1}$, was significantly higher than that in the normal subjects ($p<0.05$) (Figure 7.3). Correcting for rate-pressure product which is a rough approximation of myocardial oxygen consumption (the main determinant of basal MBF), there was no significant difference in basal MBF. Following dipyridamole infusion, MBF in the remote regions increased to 2.89±0.93 $ml \cdot min^{-1} \cdot g^{-1}$, which was greater than in the ischaemic territory ($p<0.01$) (Figure 7.3), but lower than that in normals, 3.65±0.94 $ml \cdot min^{-1} \cdot g^{-1}$, ($p<0.05$). Thus, although coronary vasodilator reserve in the remote regions, 2.73±0.89, was greater than in the ischaemic territory ($p<0.01$ vs. ischaemic region), it was significantly lower than that in the normal subjects, 4.07±0.98 ($p<0.01$ vs remote region) (Figure 7.4).

ii) Myocardial Metabolism

(Tables 7.4, 7.5 and 7.6)

At baseline, haemodynamic parameters and myocardial substrate utilisation were similar to those in the controls (Tables 7.4 and 7.5). All the group 2b patients

Table 7.4. Global and Regional Left Ventriculography in Group 2 Patients at Basal and at Maximal Pacing

		Basal State						Maximal Pacing					
			Regional wall motion (% of contribution)						Regional wall motion (% of contribution)				
Patients	Stenosis-related artery	LV ejection fraction (%)	Antero-basal	Antero-lateral	Apical	Inferior	Postero-basal	LV ejection fraction (%)	Antero-basal	Antero-lateral	Apical	Inferior	Postero-basal
Group 2a													
1	LAD	88.3	23.3	17.6	6.5	17.8	23.1	54.2	16.4	8.8	0.5	11.6	17.0
2	LAD	86.6	23.0	17.3	3.8	19.3	23.2	69.1	19.2	12.6	3.1	17.1	17.2
3	LAD	69.7	15.4	12.8	4.2	15.8	21.5	55.4	15.6	4.9	2.5	14.6	17.9
4	LAD	91.9	24.3	16.7	5.2	17.9	27.9	67.9	17.9	5.1	1.8	13.3	27.9
5	LAD	72.4	18.6	13.2	4.2	17.4	19.1	62.8	12.9	9.4	1.6	19.4	19.5
Mean±SD		81.8±10.1	20.9±3.6	15.5±2.5	4.8±1.1	17.6±1.3	23.0±3.1	61.9±6.9†	16.4±2.5*	8.2±3.1†	1.9±1.1*	15.2±3.1	19.9±4.7
Group 2b													
6	RCA	69.1	20.8	13.7	2.4	14.4	17.9	62.2	19.7	14.9	5.6	14.9	7.4
7	RCA/LCX	63.1	18.4	15.0	2.9	13.2	13.6	60.8	19.3	16.0	3.8	7.7	14.0
8	RCA	74.4	21.0	14.0	4.8	14.4	20.2	55.8	21.8	14.9	4.0	5.8	9.3
9	RCA	83.8	18.7	20.1	5.6	18.9	20.5	-	-	-	-	-	-
10	RCA	71.5	20.1	13.4	3.7	13.5	20.8	50.0	16.7	14.7	3.3	8.0	7.2
Mean±SD		72.4±7.6	19.8±1.1	15.2±6.5	3.9±1.3	14.9±2.2	18.6±2.9	57.2±6.3*	19.4±2.2	15.1±0.7	4.2±1.1	9.1±4.5*	9.5±3.6†
Total±SD		79.0±6.9						59.8±4.5†					

* $p<0.05$ vs. Basal, † $p<0.01$ vs. Basal. LV = left ventricular; LAD = left anterior descending artery, LCX = left circumflex artery, RCA = right coronary artery. For noraml ranges of % wall motion, see Figure 8.2.

Table 7.5. Haemodynamic Variables in Group 2 Patients

Basal State

Subgroups	Heart Rate (beats·min^{-1})	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Rate-Pressure Product (mmHg·min^{-1})	LV End-Diastolic Pressure (mmHg)	Coronary Resistance (units)
Group 2a	70±10	148±17 *	74±8	10,300±1,240	6±5	1.84±0.85
Group 2b	65±6	145±13 *	73±10	9,470±1,290	10±7	1.83±0.69
Controls	76±14	124±16	74±13	9,440±2,190	6±3	1.96±0.70

Maximal Pacing

Subgroups	Heart rate (beats·min^{-1})	Systolic Blood Pressure (mmHg)	Diastolic blood pressure (mmHg)	Rate-Pressure Product (mmHg·min^{-1})	LV End-Diastolic Pressure (mmHg)	Coronary Resistance (units)
Group 2a	126±21 *	149±32	90±16	18,280±740	6±6	1.24±0.68
Group 2b	131±19	142±19	84±16	18,430±2,910	7±7	1.26±0.57
Controls	159±23	134±15	89±14	21,130±2,810	8±5	1.03±0.49

* $p<0.05$ vs. controls. LV = left ventricular.

Table 7.6. Regional Myocardial Metabolism

	Basal State			Maximal Pacing		
	Ischaemic region	Remote region	Controls	Ischaemic region	Remote region	Controls
Oxygen extraction (%)	73.0±4.0	71.6±4.1	71.2±7.4	69.1±3.0 *	67.3±6.4	63.8±7.0
Oxygen consumption ($\mu mol \cdot min^{-1}$)	400±225	362±132	301±131	650±369 *	535±190	593±174†
Respiratory quotient	0.72±0.03	0.74±0.14	0.72±0.10	0.73±0.18	0.84±0.17	0.84±0.15 *
Free fatty acid extraction (%)	19.1±15.6	31.3±12.3	30.3±11.8	25.3±15.6	20.3±11.9	22.0±12.7
Glucose extraction (%)	7.4±8.7	-0.4±7.8	1.4±3.9	8.1±5.6 §	6.4±3.3§	1.7±3.3
Lactate extraction (%)	23.8±17.1	31.0±23.5	35.6±11.9	-17.9±26.9 *§	38.2±17.0	25.7±11.1
Alanine extraction (%)	-1.0±13.5	-1.9±6.6	-6.1±3.7	6.2±6.4 §	3.6±3.1 §	-1.0±3.2†
Pyruvate extraction (%)	34.0±12.8	21.9±28.0	48.9±10.7	-28.7±88.7	20.8±26.9	32.8±18.2 *
Carbohydrate uptake ($\mu mol \cdot min^{-1}$)	21.0±12.5	4.7±19.8	11.4±13.6	30.1±26.6	44.4±20.7 *	25.8±17.6
Carbohydrate oxidation ($\mu mol \cdot min^{-1}$)	6.7±10.2	2.2±23.4	-1.1±19.3	19.8±69.2	37.4±49.6	45.0±47.6 *
Lipid uptake ($\mu mol \cdot min^{-1}$)	8.8±8.0	11.3±6.3	10.0±6.1	24.1±17.3	9.5±7.6	12.4±25.1
Lipid oxidation ($\mu mol \cdot min^{-1}$)	15.7±7.9	15.2±10.8	13.4±9.8	23.2±13.3	13.7±14.4	14.3±15.2

* $p<0.05$, † $p<0.01$ vs. Basal, § $p<0.05$ vs. controls.

developed chest pain, ≥0.1 mV ST segment depression and regional wall motion abnormalities in the inferior and/or postero-basal wall (Table 7.4), at a maximal pacing rate that was lower than in the controls (131±19 vs. 159±23 beats·min^{-1}), although this did not reach statistical significance (Table 7.5). GCV flow to the remote region increased by 59±31% which was significantly lower than the flow increment in the controls (133±66%, $p<0.05$ vs. group 2b). The metabolic data at maximal pacing are shown in Table 7.6. The extraction of glucose and alanine was significantly higher than in the controls ($p<0.05$), but there were no differences in lactate extraction, the RQ and total carbohydrate or lipid oxidation.

7.5 Discussion

This study demonstrates that, as expected, the coronary vasodilator reserve was severely reduced in regions supplied by stenotic or occluded coronary arteries. Similarly, metabolic evidence of ischaemia was found at pacing stress in comparable regions in our second patient group. In addition, a reduced coronary vasodilator reserve and changes in myocardial metabolism during stress were found in myocardial regions subtended by angiographically normal arteries. These results suggest that, even in the absence of previous infarction, regions remote from ischaemic areas may be altered functionally.

The reduction of the coronary vasodilator reserve in the remote non-ischaemic regions was due to the combination of a higher basal myocardial blood flow and a reduced vasodilator response following dipyridamole infusion, compared with controls. The higher basal myocardial blood flow in the remote region was associated with a higher resting rate-pressure product, and after correction for the rate-pressure product, there was no significant difference in basal myocardial blood flow when comparing control and remote regions. In agreement with our findings, a reduced

coronary vasodilator reserve after dipyridamole in remote areas supplied by angiographically normal arteries in patients without previous infarction, has recently been reported by others (Sambuceti et al, 1991; Beanlands et al, 1992).

Metabolism in the remote myocardium was characterized by an increased utilisation of carbohydrate fuel at maximal stress similar to that in the ischaemic region (Camici et al, 1991). This is consistent with previous work which demonstrated an increase in myocardial ^{18}F-fluoro-deoxyglucose uptake in regions subtended by non-stenotic arteries in patients with coronary artery disease and angina (Araujo et al, 1988). However, while carbohydrates are used anaerobically in the ischaemic region, as proven by the net lactate release, they are mainly oxidised in the remote regions similarly to normal controls.

Mechanisms for Alterations in Remote Metabolism and Vasodilatation

The changes that occur in the remote non-ischaemic areas could be due to different mechanisms which are not necessarily mutually exclusive. The metabolic pattern seen in remote non-ischaemic areas is in agreement with previous observations by Liedtke et al (Liedtke et al, 1982), who reported increased glucose utilisation in areas adjacent to ischaemic myocardium in isolated pig hearts. This could be due to the increased workload on the remote regions to compensate for the depressed contractile function in the ischaemic zones (Liedtke et al, 1982). In this respect, it is of note that equicaloric replacement of lipid oxidation by carbohydrate oxidation saves 0.17 $\mu mol \cdot min^{-1}$ of oxygen consumption per joule of energy produced, giving greater energy production for the same oxygen consumption (Ferrannini, 1988). This hypothesis is consistent with previous experimental studies which demonstrate that acute coronary occlusion results in enhanced contractile function in the remote non-ischaemic zone (Naccarella et al, 1984; Smalling et al, 1986; Buda et al 1990). This might be a consequence of a compensatory contractile response of the remote non-ischaemic regions for the

temporary dysfunction in the regions subtended by stenosed arteries during episodes of ischaemia. This could occur through increased activity of the sympathetic nervous system, which is known to affect contractility as well as carbohydrate metabolism (Camici et al, 1989a). In addition, the increased sympathetic drive could lead to α-adrenergic coronary vasoconstriction (Heusch, 1990) with a reduction in the coronary vasodilator reserve.

There are other possible explanations for these findings. The impairment in coronary vasodilator reserve may be a more chronic adaptation to the increased load on the remote regions with structural remodelling of the coronary vasculature as has been described in arterial hypertension (Marcus et al, 1983; Folkow, 1990). Finally, the impairment in coronary vasodilator reserve may also be an early manifestation of microvascular endothelial dysfunction in the presence of angiographically undetectable coronary atherosclerosis (Chapter 1, Section 1.5). As described above, the atherosclerotic process causes early abnormalities in coronary microvessels manifest as impaired endothelial-dependent vasodilatation to acetylcholine in an angiographically smooth artery in patients with disease elsewhere (Zeiher et al, 1991). There is evidence in an animal model (Kuo et al, 1992) and in man (Drexler et al, 1991) that L-arginine infusion may restore the vasodilator response implying deficient EDRF production or release as the cause of this abnormality. These findings could also explain the impaired coronary vasodilator reserve described in this study.

The latter explanation is not necessarily mutually exclusive from an increase in sympathetic activation. Recent work in the beating dog heart model has shown that coronary microvascular vasoconstriction by both α_1- and α_2-adrenergic activation may be increased by inhibition of nitric oxide synthesis (Jones et al, 1993) (Chapter 1, Section 1.4.2.1), implying that nitric oxide release modulated resting coronary microvascular tone, opposing the constrictor action of catecholamines. It may be that with vasodilatation either through pharmacological means, pacing stress or exercise,

this imbalance persists such that EDRF release is either insufficient or the responsiveness of the vascular smooth muscle is reduced such that α-adrenergic vasoconstriction predominates to diminish maximal vasodilatation, and an unopposed increase in β-adrenergic activation acts to stimulate myocyte carbohydrate uptake. This hypothesis could unify the impaired vasodilator response and altered metabolism seen in this study.

Potential Limitations

Some important considerations may limit the conclusions that can be derived from this preliminary study. Firstly, the control subjects studied by dynamic PET were significantly younger, compared to the patient group, and it could be argued that coronary vasodilator reserve is reduced with age. However, in a recent study by Geltman et al (Geltman et al, 1990) using $^{15}H_2O$ and PET, the coronary vasodilator reserve to intravenous dipyridamole (0.56 $mg \cdot kg^{-1}$), measured in 9 patients of mean age 54 years (range 40-73) with normal or minimally diseased coronary arteries, was 4.4±0.4. This was not significantly different from a coronary vasodilator reserve of 3.8±0.3 measured in 16 normal controls of mean age 25 years (range 20-35). Similar values of coronary vasodilator reserve to dipyridamole have been recently reported in control subjects ranging from 18 to 84 years of age by Chan et al (Chan et al, 1992). Any reduction in coronary vasodilator reserve which does occur with age tends to occur after the age of 60 years, with an increasing exponential reduction in normal subjects in their 8th (70-79 years) and 9th (80-89 years) decades (Uren et al, unpublished observations). Furthermore, in our study, a significantly reduced increment in great cardiac vein flow during pacing was also seen in the group 2b patients when compared with control subjects of similar age. One potential limitation is that the PET scanning and invasive studies were performed in two different patient groups, although their clinical characteristics were very similar. It is unlikely that the

use of different study groups significantly prejudiced results as the principal findings of the study relate most to the comparison of normal and abnormal myocardial regions with patients thus acting as their own controls.

A potential source of error relates to the accuracy in defining a myocardial region of interest as truly representative of the area under study. The complicating variable here would be the inclusion of "watershed" areas of normal myocardium contaminating the data from the study region. However, in addition to great care being taken to define the myocardial region carefully in relation to the coronary anatomy derived at angiography, such an error would only occur to diminish the difference between the study region and remote myocardium. An error of potentially greater concern is the reverse contamination of remote myocardium by ischaemic or otherwise abnormal myocardium. To avoid this problem, sampling was done high in the great cardiac vein to avoid contamination from venous drainage from ischaemic regions (in the patients with "normal" left anterior descending arteries). In all PET studies reported in this thesis, the remote region of interest was always diametrically opposite the study region.

This problem of what is normal persists despite careful data sampling and analysis. Intravascular ultrasound has shown that arteries that appear smooth at coronary angiography may already have evidence of intramural atherosclerosis before the development of intraluminal plaque (St. Goar et al, 1992). This does not invalidate the observations made above given that this recent technique characterises the state of the epicardial artery alone, irrespective of resistive vessel function. However, it does mean that an angiographically normal epicardial artery, when disease is manifest in another vessel at angiography, probably has subclinical epicardial disease as well.

Conclusions

The coronary vasodilator reserve is reduced in remote non-ischaemic regions of myocardium in patients with angina and stable single vessel coronary artery disease, which correcting for rate-pressure product is predominately due to a reduction in the vasodilator response to dipyridamole. Increased glucose and alanine extraction also occurs in these remote regions under pacing stress in the absence of objective evidence of myocardial ischaemia, such as lactate production. These alterations in remote regions, if confirmed, may have important implications for the management of patients with coronary artery disease. In particular, it is possible that some of the beneficial effects of drugs such as beta blockers and calcium antagonists may be explained by some effect on myocardial blood flow and metabolism remote from the site of transient ischemia. Thus, preservation of coronary vasodilator reserve and myocardial metabolism in remote myocardium may be seen as an additional goal in the treatment of patients with coronary artery disease.

CHAPTER 8. ALTERED CORONARY RESISTIVE VESSEL FUNCTION IN INFARCTED AND REMOTE MYOCARDIUM AFTER MYOCARDIAL INFARCTION

8.1 Abstract

Coronary resistive vessel function may be impaired in patients with coronary artery disease, even in regions subtended by an angiographically normal artery. Thirteen patients of mean age 62±11 years were studied after thrombolysis for myocardial infarction (11 had patent infarct-related arteries and were included in a complementary study described below). Using positron emission tomography (PET) with $H_2{}^{15}O$, regional myocardial blood flow (MBF) and the coronary vasodilator reserve to intravenous dipyridamole (0.5 $mg \cdot kg^{-1}$ over 4 minutes) in infarct and remote regions was assessed, 8±3 days after infarction (n=13; PET_1) and 6±2 months after infarction (n=9; PET_2). At PET_1, basal myocardial blood flow (MBF) was 0.81±0.22 $ml \cdot min^{-1} \cdot g^{-1}$ and peak MBF was 0.91±0.51 $ml \cdot min^{-1} \cdot g^{-1}$ in the infarct region, giving a coronary vasodilator reserve of 1.12±0.50. Basal MBF was 1.09±0.32 $ml \cdot min \cdot g^{-1}$ ($p<0.05$ vs. infarct region) and peak MBF was 1.70±0.72 $ml \cdot min^{-1} \cdot g^{-1}$ at peak ($p<0.01$ vs. infarct region) in the remote region, giving a coronary vasodilator reserve of 1.53±0.36 in the remote region ($p<0.05$ vs. infarct region). The coronary vasodilator reserve in remote myocardium was significantly less than that of remote regions, subtended by a normal artery, in a control group of patients with single vessel coronary artery disease, 3.17±0.72 ($p<0.01$). At PET_2, basal MBF was 0.82±0.21 $ml \cdot min^{-1} \cdot g^{-1}$ and peak MBF was 1.20±0.45 $ml \cdot min^{-1} \cdot g^{-1}$ in the infarct region ($p<0.01$), and 1.09±0.18 $ml \cdot min^{-1} \cdot g^{-1}$ at basal and 2.38±0.89 $ml \cdot min^{-1} \cdot g^{-1}$ at peak ($p<0.01$ vs. infarct region, $p<0.05$ vs. remote region at PET_1) in the remote region. Thus, the coronary vasodilator reserve was 1.42±0.37 in the

infarct region and 2.19±0.69 in the remote region ($p<0.01$ vs. infarct region, $p<0.05$ vs. remote region at PET_1), a value which remained lower than in controls ($p<0.05$).

With the addition of four other patients, a total of 15 patients (mean age 61±12 years) with patent infarct-related arteries underwent PET scanning early after infarction. Regional MBF and the perfusable tissue index (PTI), a measure of myocardial viability, was measured. The PTI correlated directly with the MBF response to dipyridamole ($R=0.58$, $p<0.05$) and negatively with the time to thrombolysis ($R=-0.60$, $p<0.05$). No relationship was seen between the coronary vasodilator reserve in the infarct region and the residual coronary stenosis.

In conclusion, there is marked impairment of the coronary vasodilator reserve in the infarct region early after infarction with a small recovery by 6 months. This impaired response to dipyridamole is directly proportional to the viability of the tissue in the infarct region. The coronary vasodilator reserve to dipyridamole is acutely decreased in regions remote from the site of infarction compared to remote regions in stable patients with single vessel disease. This impairment in the coronary vasodilator reserve improves by 6 months after infarction, but remains lower than in stable patients.

8.2 Introduction

Coronary blood flow in viable myocardium subtended by infarct-related arteries may remain severely impaired despite successful recanalisation (Kloner et al, 1974; Willerson et al, 1975; Jeremy et al, 1990). This observation could be explained by resistive vessel dysfunction occurring in infarcted tissue as a consequence of acute ischaemia, but particularly at reperfusion because of neutrophil and platelet accumulation with progressive impairment of post-ischaemic flow (Ambrosio et al, 1989) and marked microvascular endothelial dysfunction due to oxygen free radical

production at reperfusion (Quillen et al, 1990). These processes could account for the wide variability described in the coronary blood flow and the coronary vasodilator reserve in the infarcted region (Kloner et al, 1974; Schofer et al, 1985).

Recent studies have indicated that in patients with chronic stable angina, basal coronary blood flow and the coronary vasodilator reserve may also be altered in regions of myocardium perfused by angiographically normal arteries distant from the region of ischaemia (Uren et al, 1993b; Sambuceti et al, 1991; Beanlands et al, 1992), probably as a result of resistive vessel dysfunction due to atherosclerosis, as suggested by several studies (Cox et al, 1989; McLenachan et al, 1991; Sellke et al, 1990; Zeiher et al, 1991). It is possible that coronary resistive vessel dysfunction may be accentuated in the remote region subtended by an angiographically normal artery after acute myocardial infarction.

To study coronary resistive vessel function in patients after acute myocardial infarction, we used dynamic positron emission tomography to measure regional myocardial perfusion and the coronary vasodilator reserve in infarct regions, and remote regions of myocardium subtended by angiographically normal arteries after thrombolysis for transmural myocardial infarction, both in the first few days after myocardial infarction and at several months follow-up. In addition, the relationship between the coronary vasodilator reserve and myocardial viability, described by the perfusable tissue index (Chapter 3, Section 3.9), in the infarct region was investigated using this technique.

8.3 Methods

8.3.1. Patient Population

A total number of 17 patients with single vessel disease were studied after myocardial infarction. Patients with multiple vessel disease or those who developed

recurrent myocardial ischaemia at rest, acute heart failure or required inotropic support were excluded from the study. Thirteen consecutive patients (mean age 62±11 years, range 40-77; 10 male) with single vessel disease were studied initially, in whom all had left ventriculography after myocardial infarction. Of this group, 11 of 13 patients had a patent infarct-related artery defined as TIMI grade 2 or 3 (Table 8.1) (TIMI group, 1985). The infarct-related artery was the left anterior descending (LAD) in 8 patients, a dominant right coronary artery (RCA) in 4 patients and a dominant left circumflex artery (LCX) in 1 patient. All other arteries were angiographically normal. All patients received intravenous streptokinase (1.5 MU) and soluble aspirin 300 mg, 4.2±2.4 hours (range 2.0-7.8) after the onset of chest pain (Results, Table 8.1).

Ten male patients (mean age 52±9 years, range 44-72) with chronic stable angina due to single vessel (LAD) coronary artery disease were studied as a control group. All had normal left ventricular function and no evidence of previous myocardial infarction. All other arteries were angiographically normal.

In addition, to study the relationship between the severity of the residual coronary stenosis, coronary vasodilator reserve and myocardial viability in the infarct zone, an additional four patients (3 male) of mean age 58±13 years, who had coronary arteriography alone (without left ventriculography) after infarction with the demonstration of a single open infarct-related artery were studied (Section 8.5.7). These data were combined with that of the 11 patients with open-infarct related arteries from the initial study group to study the relationship between coronary stenosis severity and myocardial blood flow (Section 8.4, Table 8.1).

8.3.2. Study Protocol

The protocol was approved by the Research Ethics Committee of Hammersmith Hospital and all patients gave informed and written consent.

i) Myocardial Infarction Patients.

None of the 13 patients studied had been given either ß-blockers or calcium antagonists after myocardial infarction. PET scanning was performed 8±3 days (range 4-13 days) after myocardial infarction (PET_1) and cardiac catheterisation was done 27±35 days (range 7-122 days) after myocardial infarction. Patients underwent repeat PET scanning (PET_2) 6±2 months after myocardial infarction. Prior to PET_2, any anti-anginal medication (except sublingual nitroglycerin) was discontinued for at least 72 hours. No patient took nitroglycerin within 12 hours of any part of the protocol, and all abstained from drinking tea or coffee on the morning of the PET scan. Treadmill exercise testing was performed according to the modified Bruce protocol.

Of the additional 4 patients studied, PET scanning was performed 4 days (range 3-6) after myocardial infarction and coronary arteriography was done 3 days (range 1-6) after myocardial infarction. The same restrictions in abstinence from medication applied to this group also.

ii) Controls

The control group were enrolled from the routine cardiac catheterisation list. Discontinuation of anti-anginal medication and abstinence from theophylline-containing compounds was observed as had been done for the study patients.

8.3.2.1. Cardiac Catheterisation and Quantitative Coronary Arteriography

Coronary arteriography was performed as clinically indicated in order to best demonstrate the lesion morphology. Coronary arteriograms were analysed by an automated edge contour detection computer analysis system (Cardiovascular Angiographic Analysis System [CAAS], Pie Medical Equipment BV, Maastricht, The Netherlands) (Reiber et al, 1984). This method is briefly described in Chapter 5

(Section 5.3.2.2). Stenosis severity was expressed as percentage reduction of the internal luminal diameter (or area) relative to the estimated diameter interpolated from the diameters at the proximal and distal boundaries of the stenosis, as well as the absolute minimal luminal diameter and area (Reiber et al, 1984). Infarct artery patency was defined according to the TIMI grading of re-canalisation after myocardial infarction (TIMI group, 1985).

Global and regional left ventricular function was measured from the 30° right anterior oblique left ventricular cine-angiogram excluding the posterior papillary muscle with an automated hard-wired endocardial contour detector linked to a microcomputer (Serruys et al, 1986; Slager et al, 1979). Regional contribution was described in 5 territories; antero-basal, antero-lateral, apical, inferior, and postero-basal (Serruys et al, 1986). In patients with anterior or antero-lateral infarction, the postero-basal and inferior regions were considered remote; in patients with inferior infarction, the antero-basal and antero-lateral regions were considered remote from the infarct region.

8.3.2.2. Positron Emission Tomography

All PET scans were performed with an ECAT 931-08/12 camera (CTI Inc., Knoxville, Tennessee, USA). Regional myocardial blood flow (MBF, in $ml.min^{-1}.g^{-1}$) was measured in patients and controls using oxygen-15-labelled water ($H_2{}^{15}O$) as a flow tracer using the previously validated $C^{15}O_2$ inhalation technique (Araujo et al, 1991). Measurements were made at rest and 2 minutes after intravenous administration of dipyridamole (0.5 $mg \cdot kg^{-1}$ over 4 minutes). Heart rate, systemic blood pressure and a 12 lead ECG were recorded every minute during the dipyridamole infusion and for up to 10 minutes after the end of infusion. Images were created and analysed as previously described (Araujo et al, 1991; Spinks et al, 1988).

In patients with the LAD as the infarct-related artery, the anterior region was designated as the infarct region and the infero-posterior region as remote. In patients with the RCA or LCX as the infarct-related artery, the infero-posterior region was designated as the infarct region and the anterior region as remote. Basal myocardial blood flow (MBF) and peak MBF after dipyridamole were determined. The coronary vasodilator reserve was defined as the ratio of peak MBF to basal MBF. In the control subjects, the infero-posterior region was defined as the remote region as all had single vessel LAD disease.

To exclude the effect of changes in mean arterial pressure (and thus perfusion pressure) on coronary flow, regional coronary vascular resistance was calculated as mean arterial pressure÷MBF both under basal conditions and after dipyridamole, and expressed as $mmHg \cdot min \cdot g \cdot ml^{-1}$.

8.3.3. Statistical Analysis

All data are expressed as mean±SD. Paired Student's t tests were used to compare mean group values. Simultaneous comparison of more than two mean values was performed using one-way analysis of variance (ANOVA), and Fisher's least significant difference method was subsequently applied to localize the source of the difference (Godfrey et al, 1985). Correlations between parameters were examined using simple linear regression. A p value <0.05 was considered statistically significant.

8.4 Results

8.4.1. Clinical Characteristics of Myocardial Infarction/

8.4.1. Clinical Characteristics of Myocardial Infarction

(Table 8.1)

All patients developed pathological Q waves on 12 lead ECG within 24 hours of the onset of chest pain. Peak creatine kinase level was 2,733±1,914 $IU \cdot l^{-1}$. At exercise testing at 8±3 days after infarction, 6 of 13 patients had a positive exercise test (defined as ≥0.1 mV ST segment depression). At the time of the repeat PET study, 7 of 9 patients had a positive exercise test.

8.4.2. Regional Left Ventricular Function

(Table 8.2)

The mean left ventricular ejection fraction was 56.1±9.8% and mean left ventricular end-diastolic pressure was 17±4 mmHg at left ventriculography. At regional left ventricular analysis (Section 8.3), only one segment in the remote regions demonstrated an increased regional contribution to left ventricular function away from the designated region of myocardial infarction (patient 1, anterolateral). An additional segment with increased regional contribution to global function (patient 9, anterobasal) was demonstrated in a patient with the LAD as the infarct-related artery, although this patient had no regional impairment of contractile function (Table 8.2).

8.4.3. Quantitative Coronary Arteriography

(Table 8.3)

Eleven of 13 patients had successful recanalisation of the infarct-related artery (TIMI grade 3 in 10 patients) with a mean residual stenosis of minimum luminal diameter of 0.72±0.37 mm, equivalent to a minimum luminal area of 0.60±0.53 mm^2. Compared with the interpolated reference diameter of 2.75±0.55 mm (an interpolated

Table 8.1. Patient Characteristics

Pt. Nos.	Age/sex	Q waves	Infarct-related artery	CK peak *(IU·l^{-1})*	Time to thrombolysis *(hours)*	ETT_1	ETT_2
1	50/M	III	RCA	484	3.5	-	0
2	60/M	III, aVF	LCX	5,200	4.0	+	0
3	71/F	III, aVF	RCA	6,102	2.8	+	0
4	76/M	II, III, aVF	RCA	573	2.0	+	+
5	40/M	V2-5	LAD	2,885	8.5	-	-
6	52/M	V1-3	LAD	1,452	2.8	-	+
7	55/M	V1-3	LAD	3,020	3.3	+	+
8	59/M	V1-5	LAD	4,260	2.5	-	+
9	61/M	V1-3	LAD	549	2.5	+	+
10	65/F	V1-5	LAD	4,148	6.5	-	0
11	67/M	V1-4	LAD	1,265	2.5	-	-
12	70/M	V1-3	LAD	1,422	6.2	-	+
13	78/M	V1, 2	LAD	4,170	7.8	+	+
Mean±SD	62±11			2,733±1,914	4.2±2.4		
14	69/M	I, aVL, V1-6	LAD	2,302	2.4	0	0
15	67/F	V1-3	LAD	6,100	1.8	0	0
16	55/M	II, III, aVF	RCA	2,900	3.3	+	0
17	40/M	II, III, aVF	LCX	593	1.8	-	0

CK = creatine kinase, ETT = exercise treadmill test (+ = positive, - = negative, 0 = no test), LAD = left anterior descending artery, LCX = dominant left circumflex artery, RCA = dominant right coronary artery.

Table 8.2. Global and Regional Left Ventriculography

			Regional wall motion (% of contribution)				
Patients	Infarct-related artery	LV ejection fraction (53.3-92.6%)	Antero-basal (14.4-25.4%)	Antero-lateral (10.1-17.3%)	Apical (2.5-6.3%)	Inferior (10.6-18.2%)	Postero-basal (15.7-25.4%)
1	RCA	62.7	18.5	(19.5)	3.2	12.5	9.0
2	LCX	52.1	20.0	17.1	**2.2**	**4.8**	**8.0**
3	RCA	59.9	21.0	16.2	**1.2**	12.6	**8.9**
4	RCA	58.8	22.9	16.8	**1.7**	**5.3**	**12.0**
5	LAD	69.7	15.7	**9.6**	3.3	18.2	23.0
6	LAD	48.5	**12.6**	**0.8**	**2.3**	13.3	19.5
7	LAD	54.2	**13.3**	**5.7**	2.8	12.4	14.8
8	LAD	55.0	16.0	**7.8**	**1.3**	11.7	18.5
9	LAD	74.4	(29.7)	17.2	5.0	12.7	16.6
10	LAD	54.5	16.9	**5.8**	**-5.0**	**9.6**	22.7
11	LAD	48.0	**9.0**	**7.0**	2.9	14.4	20.2
12	LAD	52.4	**9.8**	**6.3**	**0.6**	13.9	21.8
13	LAD	37.9	21.1	**10.1**	**-1.5**	**-1.0**	**9.2**

Hyperkinetic segments in parenthesis, hypokinetic segments in bold. Normal ranges given in parentheses. LV = left ventricular; LAD = left anterior descending artery, LCX = dominant left circumflex artery, RCA = dominant right coronary artery.

Table 8.3. Quantitative Coronary Arteriography

Patients	Infarct-related artery	TIMI grade	Minimum luminal diameter (mm)	Reference diameter (mm)	Minimum luminal area (mm^2)	Reference area (mm^2)	Diameter stenosis (%)	Area stenosis (%)
1	RCA	3	0.83	3.08	0.54	7.45	73.1	92.8
2	LCX	3	0.97	3.20	0.74	8.04	69.7	90.8
3	RCA	3	1.46	3.48	1.67	9.51	58.0	82.4
4	RCA	3	0.44	2.92	0.15	6.69	84.9	97.8
5	LAD	2	0.40	1.61	0.13	2.03	75.2	93.6
6	LAD	3	0.31	2.16	0.08	3.66	85.6	97.8
9	LAD	3	0.71	2.85	0.40	6.38	75.1	93.7
10	LAD	3	0.28	2.09	0.06	3.43	86.6	98.2
11	LAD	3	1.13	3.57	1.00	10.00	68.3	90.0
12	LAD	3	0.80	2.05	0.50	3.30	61.0	84.8
13	LAD	3	1.28	3.20	1.29	8.04	59.8	78.0
14	LAD	3	0.69	2.69	0.61	5.69	74.3	89.3
15	LAD	3	2.48	3.84	2.04	11.56	35.4	82.4
16	RCA	3	1.22	2.95	1.17	6.85	59.0	82.9
17	LCX	3	0.58	2.07	0.43	3.38	72.0	87.3
Mean±SD*			0.86±0.56	2.78±0.66	0.65±59	6.40±2.81	70.6±13.3	90.7±5.7

Patients 7 and 8 are excluded from the table as they did not undergo successful recanalisation after thrombolysis; thus, mean values are derived from the 11 patients with successful recanalisation. LAD = left anterior descending artery, LCX = dominant left circumflex artery, RCA = dominant right coronary artery.

reference area of 6.23±2.72 mm^2), this gave a residual diameter stenosis of 76.3±13.8%, equivalent to an area stenosis of 92.2±6.9%.

8.4.4. Haemodynamic Parameters at PET Scanning

(Table 8.4)

At PET_1, heart rate increased from 66±12 to 80±15 beats·min^{-1} ($p<0.01$), systolic blood pressure increased from 108±15 to 114±14 mmHg ($p<0.01$), and thus, rate-pressure product increased from 7,145±1,712 to 9,108±2,313 mmHg·min^{-1} ($p<0.01$) from basal to peak dipyridamole (Table 4). There were no significant differences in any haemodynamic parameters comparing the control group to the patients at PET_1 and PET_2.

However, at basal in the 9 patients undergoing repeat study, systolic blood pressure (103±13 and 120±21 mmHg; $p<0.01$), mean arterial pressure (77±10 and 87±9 mmHg; $p<0.05$), and rate-pressure product (6,663±1,428 and 7,715±1,146 mmHg·min^{-1}; $p<0.05$) were lower at PET_1 compared to PET_2, respectively. Similarly, after dipyridamole, systolic blood pressure (110±13 and 130±21 mmHg; $p<0.05$), mean arterial pressure (80±10 and 93±8 mmHg; $p<0.05$), and rate-pressure product (8,779±2,335 and 10,311±1,572 mmHg·min^{-1}; $p<0.05$) were also lower at PET_1 compared to PET_2, respectively.

8.4.5. Regional Myocardial Blood Flow

(Figures 8.1, 8.2 and 8.3)

Regional myocardial blood flow in the controls and the patients at PET_1 and at PET_2 are shown in Figure 8.1. After a mean of 8 days, in the 13 patients studied, basal MBF was 0.81±0.22 ml·min^{-1}·g^{-1} and peak MBF was 0.91±0.51 ml·min^{-1}·g^{-1} in the infarct region, and 1.09±0.32 ml·min·g^{-1} at basal ($p<0.05$ vs. infarct region) and 1.70±0.72 ml·min^{-1}·g^{-1} at peak ($p<0.01$ vs. infarct region) in the remote region. Thus,

Table 8.4. Haemodynamic Variables at Positron Emission Tomography

	Patients at PET_1		Patients at PET_2		Controls	
	Basal	Dip	Basal	Dip	Basal	Dip
Heart Rate	66±12	80±15	65±10	80±15	64±11	78±15
Systolic Blood Pressure	108±15	113±14	120±21	130±21*	120±11	127±18
Diastolic Blood Pressure	66±11	68±12	71±6	74±6	72±12	74±12
Mean Arterial Pressure	80±11	83±11	87±9	93±8 *	88±11	92±13
Rate-Pressure Product	7,145±1,711	9,108±2,313	7,715±1,146	10,311±1,572	7,599±1,354	9,928±2,416

Comparison of haemodynamic variables in patients at the first PET scan (PET_1) (n=13), at the second PET scan (PET_2) (n=9), and in the control subjects (n=10). * $p<0.05$ vs. PET_1.

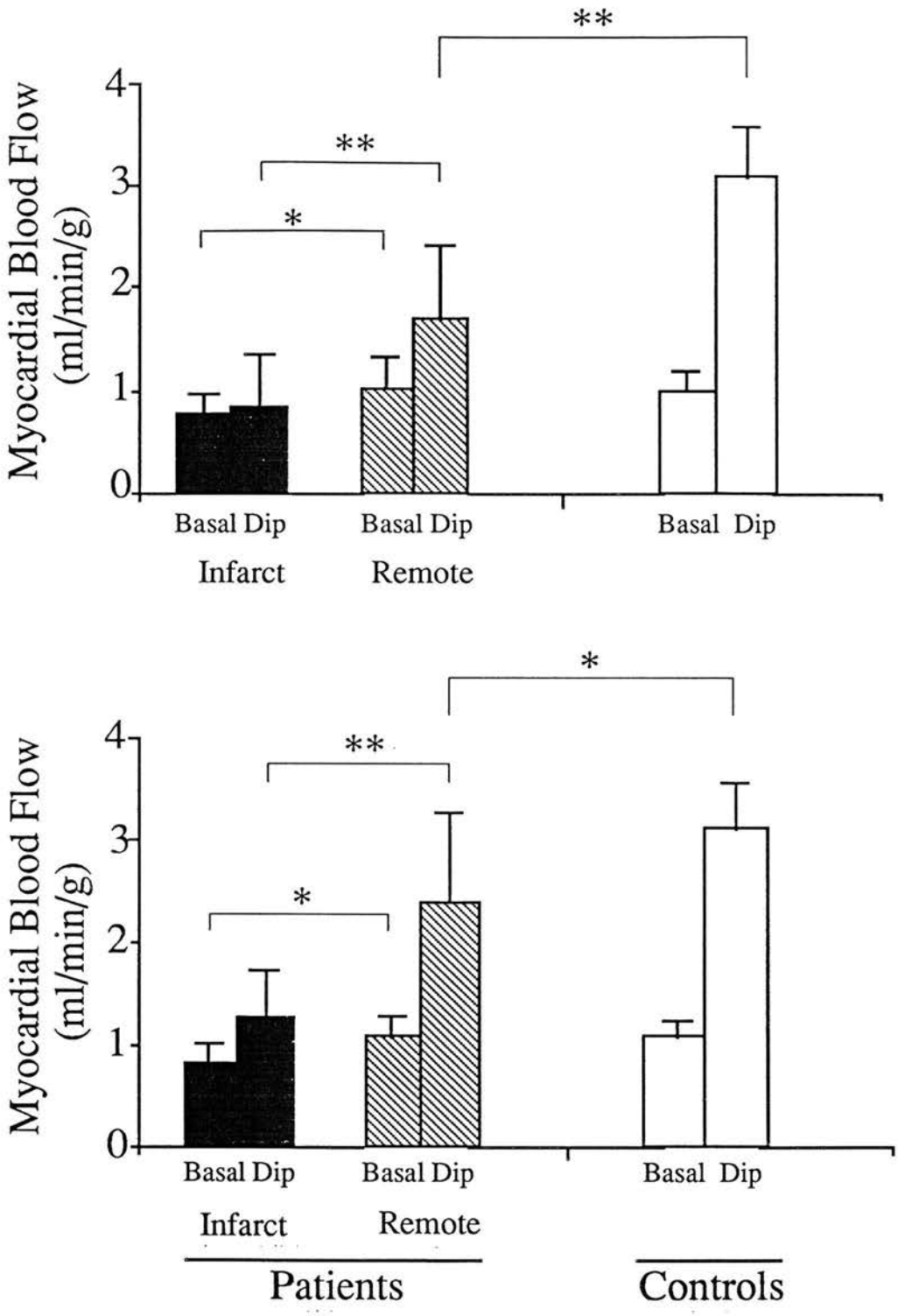


Figure 8.1. The myocardial blood flow at basal and at peak dipyridamole (Dip) in the infarct region and the remote region in the patients at PET_1 (n=13) (upper panel), and at PET_2 (n=9) (lower panel), compared to the remote region of the controls. * $p<0.05$, ** $p<0.01$.

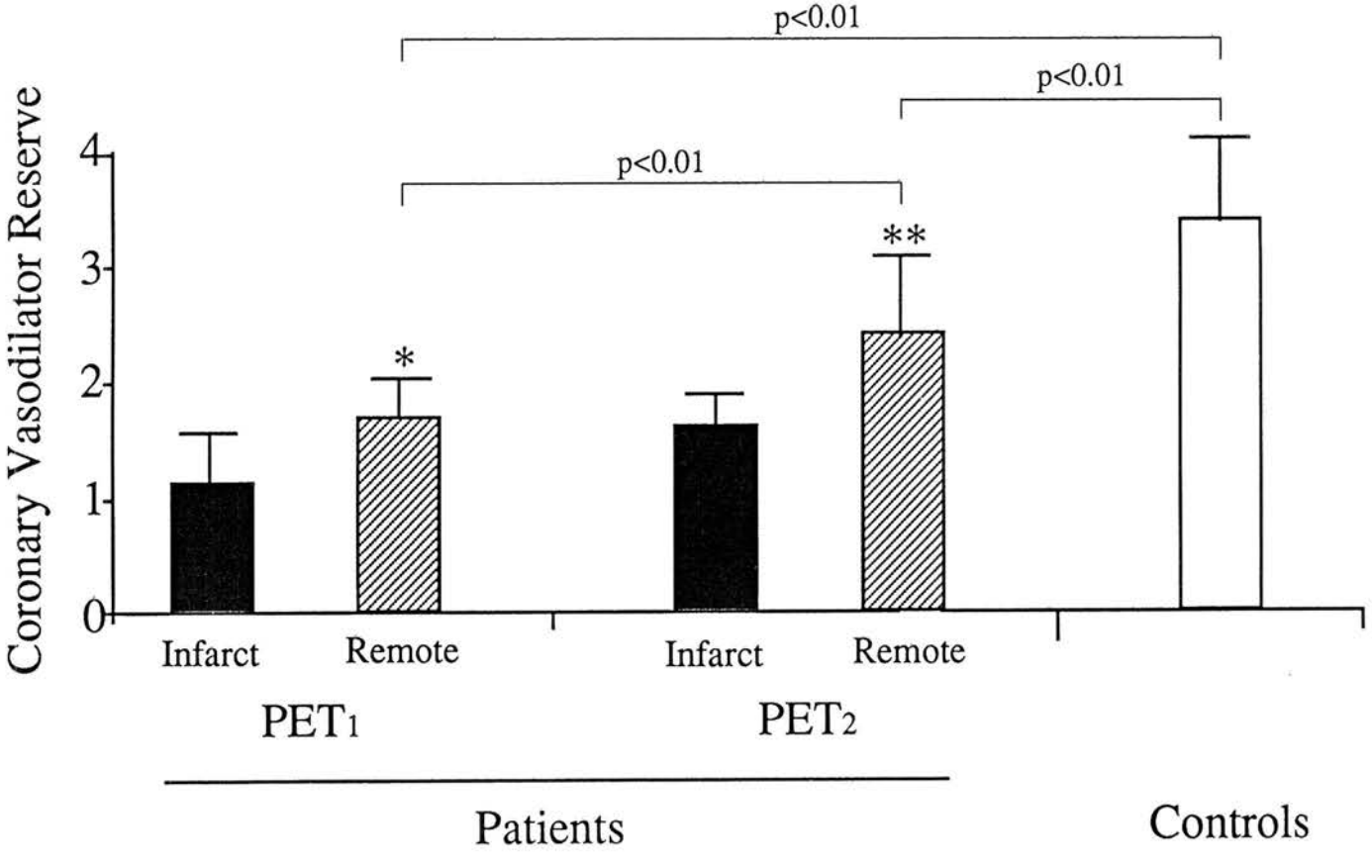


Figure 8.2. The coronary vasodilator reserve in the infarct and the remote region in the patients at PET1 (n=13) and at PET2 (n=9), compared to the remote region of the controls. * $p<0.05$, ** $p<0.01$ vs. infarct.

the coronary vasodilator reserve was 1.12±0.50 in the infarct region and 1.53±0.36 in the remote region ($p<0.05$ vs. infarct region) (Figure 8.2). In the control group, mean basal MBF was 1.00±0.16 $ml \cdot min^{-1} \cdot g^{-1}$ and the mean peak MBF was 3.08±0.53 $ml \cdot min^{-1} \cdot g^{-1}$ ($p<0.01$). Thus, the mean coronary vasodilator reserve in the remote region of the patients was reduced compared to the remote region in the control group, 3.17±0.72 ($p<0.01$ vs. remote region at PET_1) (Figure 8.2).

In the 9 patients who underwent repeat PET scanning, basal MBF was 0.82±0.21 $ml \cdot min^{-1} \cdot g^{-1}$ and peak MBF was 1.20±0.45 $ml \cdot min^{-1} \cdot g^{-1}$ in the infarct region ($p<0.01$), and 1.09±0.18 $ml \cdot min^{-1} \cdot g^{-1}$ at basal and 2.38±0.89 $ml \cdot min^{-1} g^{-1}$ at peak ($p<0.01$ vs. infarct region, $p<0.05$ vs. remote region at PET_1) in the remote region (Figure 8.1). Thus, the coronary vasodilator reserve was 1.42±0.37 in the infarct region and 2.19±0.69 in the remote region ($p<0.01$ vs. infarct region, $p<0.05$ vs. remote region at PET_1) (Figure 8.2). Comparing both PET scans, in the infarct region, there was no improvement in basal MBF (Figure 8.3).

There was no change in the mean basal MBF at 6 months, which was still significantly lower than that in the remote region. Although the response to dipyridamole did show some improvement with time, this did not reach statistical significance (Figure 8.3). In the remote regions, the peak MBF was higher at PET_2 compared to PET_1, without significant change in the basal MBF (Figures 8.2 and 8.3). However, despite this improvement, the peak MBF was lower comparing the remote region to the controls, and thus, the coronary vasodilator reserve remained lower in the remote region of the infarct patients compared to controls at PET_2 (Figure 8.2).

8.4.6. Regional Coronary Vascular Resistance

At PET_1, the coronary resistance in the infarct regions was 110.3±48.7 $mmHg \cdot min \cdot g \cdot ml^{-1}$ at basal and 118.0±61.0 $mmHg \cdot min \cdot g \cdot ml^{-1}$ after dipyridamole

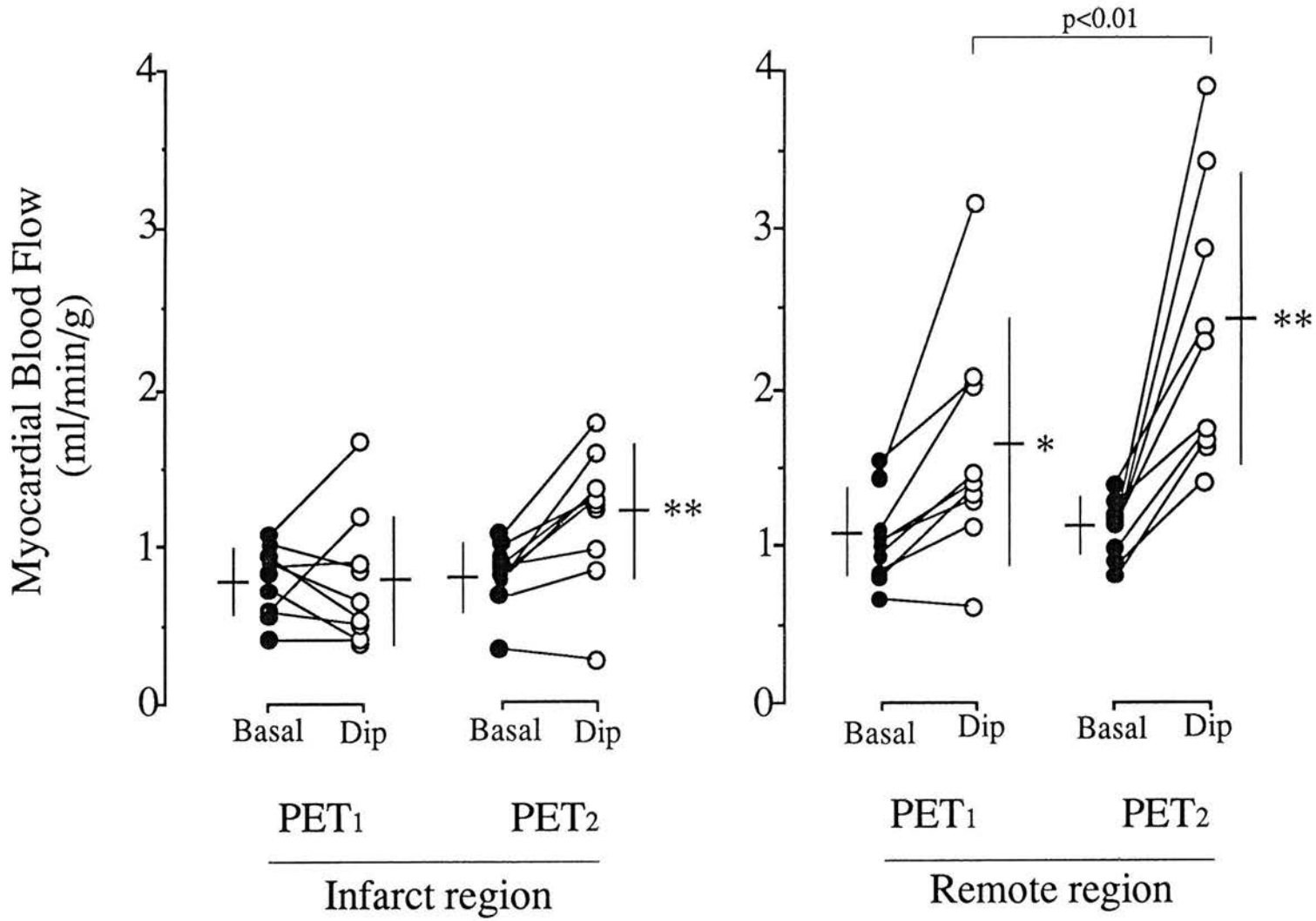


Figure 8.3. Individual values of regional myocardial blood flow at basal and after dipyridamole (Dip) in the infarct and remote region of the 9 patients undergoing repeat PET scanning. * $p<0.05$, ** $p<0.01$ vs. Basal.

(p=NS). In the remote region, it fell significantly ($p<0.01$) from 76.1±16.7 mmHg·min·g·ml^{-1} ($p<0.05$ vs. infarct region) at basal to 55.1±18.4 mmHg·min·g·ml^{-1} ($p<0.01$ vs. infarct region) after dipyridamole.

At PET_2, the coronary resistance in the infarct region was 116.9±45.8 mmHg·min·g·ml^{-1} at basal and 107.8±104.4 mmHg·min·g·ml^{-1} after dipyridamole (p=NS). In the remote region, it fell significantly ($p<0.01$) from 83.3±21.0 mmHg·min·g·ml^{-1} at basal to 43.7±14.8 mmHg·min·g·ml^{-1} after dipyridamole ($p<0.05$ vs. the remote region at PET_1). At PET_2, the coronary resistance at basal and after dipyridamole in the infarct region were not significantly different from the values in the same region at PET_1. The coronary resistance in the remote region in the controls was 90.4±6.7 mmHg·min·g·ml^{-1} at basal (p=NS vs. the remote region of the infarct patients at PET_1 and PET_2) and 30.4±6.9 mmHg·min·g·ml^{-1} after dipyridamole ($p<0.01$ vs. the remote region of the infarct patients at PET_1; $p<0.05$ vs. the remote region of the infarct patients at PET_2). Thus, at PET_2, the coronary resistance in the remote region at basal was not significantly different from that value in the same region at PET_1, although after dipyridamole the coronary resistance was significantly lower, compared with PET_1.

8.4.7. Correlates of the Coronary Vasodilator Reserve and the Perfusable Tissue Index

(Figures 8.4, 8.5, and 8.6)

In this study, 15 patients with an open infarct-related artery (TIMI grade 2/3) underwent PET scanning and coronary arteriography shortly after myocardial infarction (Table 8.1 and 8.2). The relationships between residual coronary stenosis severity, myocardial blood flow, and the perfusable tissue index was examined.

In the infarct region, no relationship was seen between i) the minimum luminal diameter, ii) percent diameter stenosis, iii) minimum luminal area, or iv) percent area

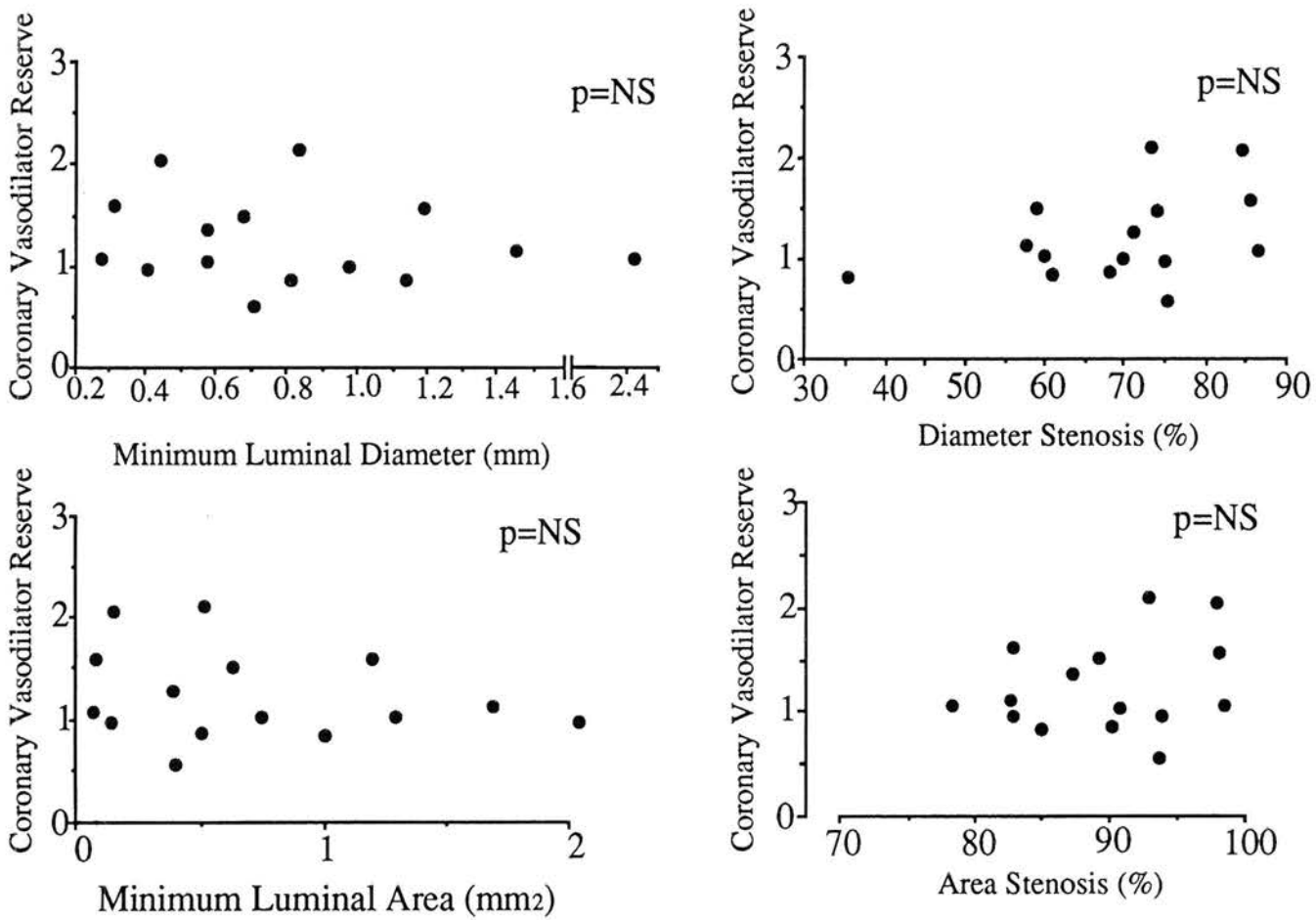


Figure 8.4. The relationship between minimum luminal diameter (upper left panel), minimum luminal area (lower left panel), percent diameter stenosis (upper right panel), and percent area stenosis (lower right panel) and the coronary vasodilator reserve in the infarct region at PET_1 in the 15 patients with open infarct-related arteries.

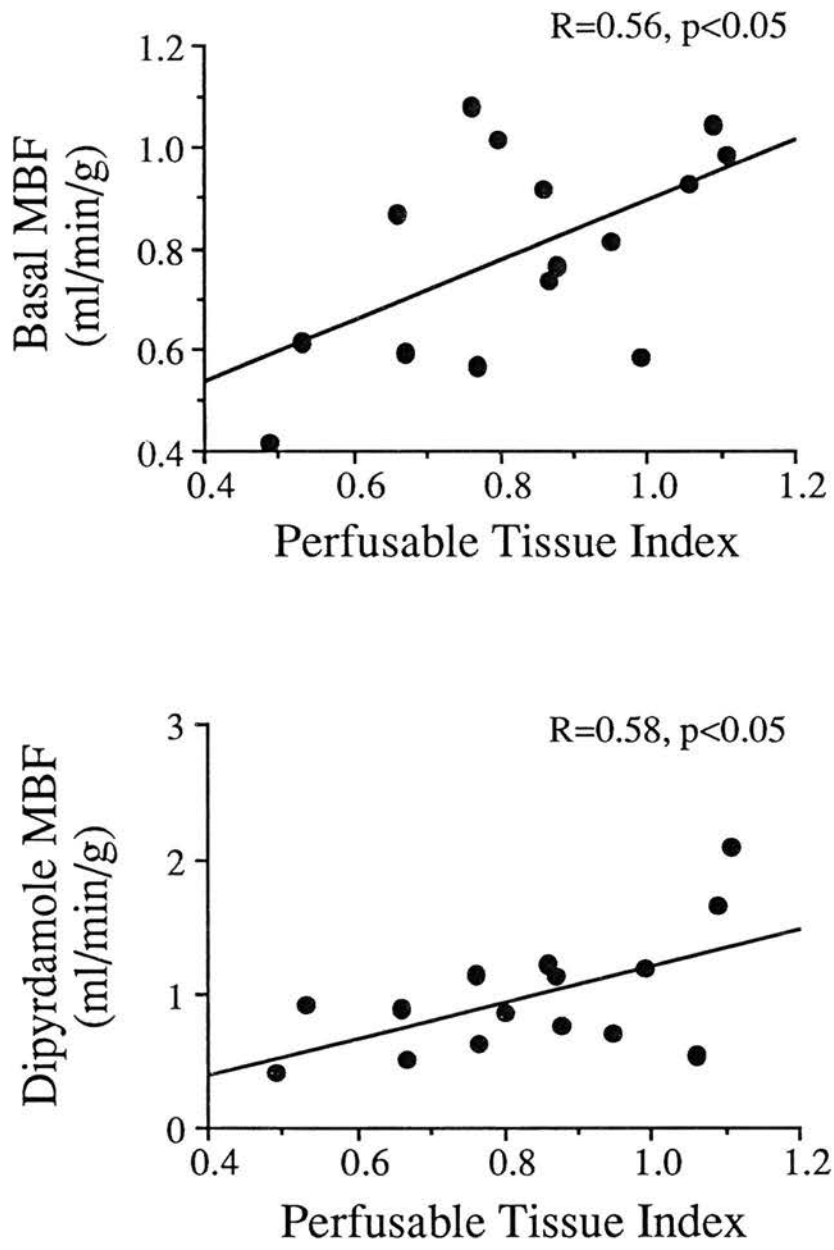


Figure 8.5. The relationship between perfusable tissue index in the infarct region and myocardial blood flow at basal in the infarct region (upper panel), and with myocardial blood flow after dipyridamole in the infarct region (lower panel), in the 15 patients with open infarct-related arteries after thrombolytic therapy.

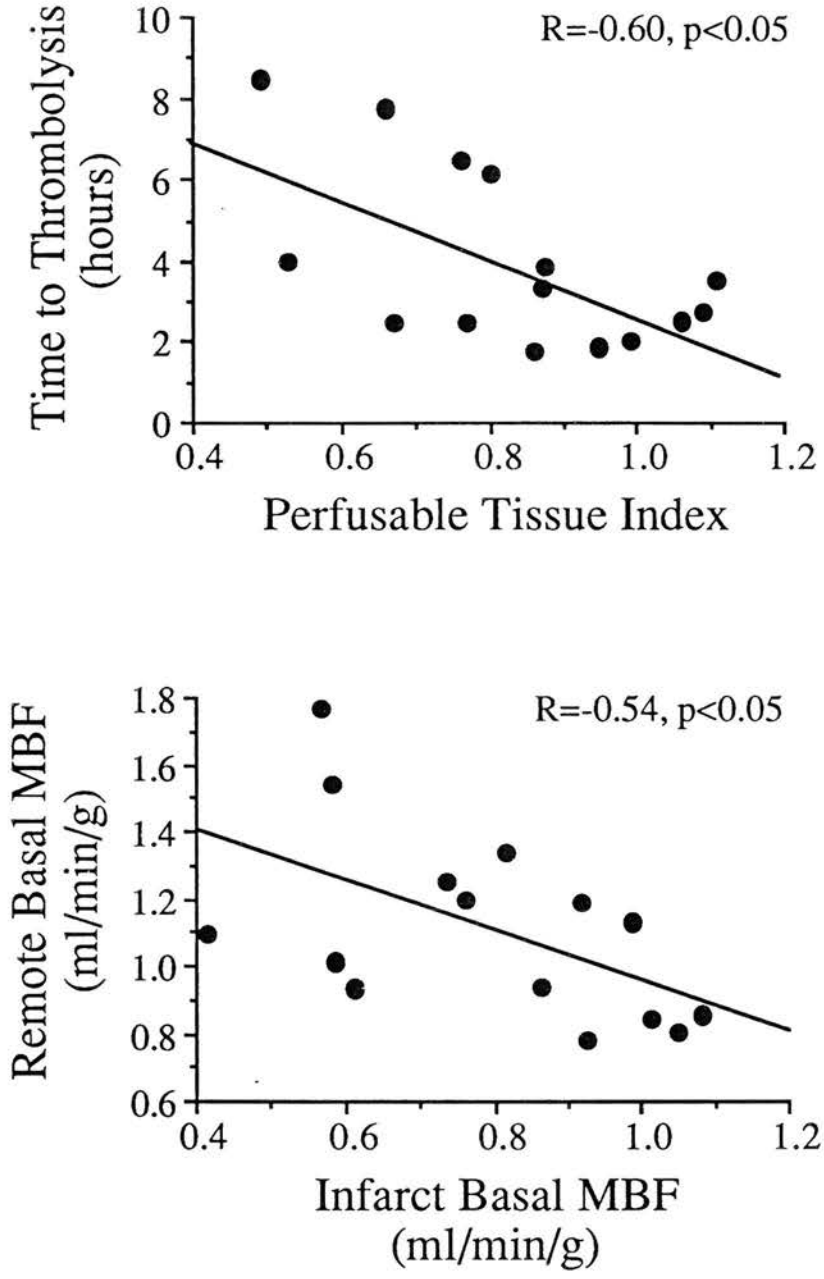


Figure 8.6. The relationship between perfusable tissue index in the infarct region and the time from onset of chest pain to initiation of thrombolysis (upper panel). In the lower panel, the relationship between basal myocardial blood flow in the infarct region and in remote myocardium is shown in the 15 patients with open infarct-related arteries after thrombolytic therapy.

stenosis and basal MBF, peak MBF after dipyridamole, or the coronary vasodilator reserve (Figure 8.4). However, in the infarct region, there was a weak but significant relationship between both basal MBF ($R=0.56$, $p<0.05$) and peak MBF ($R=0.58$, $p<0.05$) and the perfusable tissue index (Figure 8.5). This indicates that a relationship exists between the ability of residual myocardium to exchange water and basal myocardial oxygen consumption (≈basal myocardial blood flow). The residual vasodilator response to dipyridamole is also dependent on the extent of myocardial tissue still able to exchange water after infarction.

The relationship between the infarct region and the remote region were compared. The basal MBF in the infarct region correlated inversely with basal MBF in the remote region ($R=-0.54$, $p<0.05$) (Figure 8.6). No such correlation was demonstrated between the peak MBF after dipyridamole in the infarct region and that in the remote region. Nor was any relationship seen between the coronary vasodilator reserve in the infarct region and i) coronary vasodilator reserve in the remote region, ii) creatine kinase peak, and iii) time to thrombolysis, nor between the creatine kinase peak and either the coronary vasodilator reserve or the reduction in coronary resistance in the remote region. However, the perfusable tissue index was closely related to the time to thrombolysis with a negative correlation demonstrated between the two variables ($R=-0.60$, $p<0.05$) (Figure 8.6).

8.5 Discussion

This study shows that the basal myocardial blood flow was 34% lower in the infarct region compared with a remote region 8 days after acute myocardial infarction, and that the vasodilator response to dipyridamole was markedly reduced, not only in the infarct region but also to a lesser degree in the remote region. After 6 months, the basal myocardial blood flow in the infarct region was unchanged with a 46% increase

in the myocardial flow to dipyridamole. However, the coronary vasodilator reserve still remained significantly lower than the remote region. After 6 months, the basal myocardial blood flow in the remote region was unchanged and although there was some improvement in the coronary vasodilator reserve, it remained significantly lower than in the controls.

Myocardial Blood Flow in the Infarct Region

At the first PET scan, the lower values of basal myocardial in the infarct region are consistent with myocardial injury and severe resistive vessel dysfunction. There was no change in the mean basal myocardial blood flow at 6 months, which was still significantly lower than that in the remote region. Whether there was recovery of contractile function in the infarct region at follow-up, and thus evidence of myocardial stunning (Braunwald & Kloner, 1983; Bolli, 1992) in the patients with basal myocardial flows within the normal range, is not known. The persistent reduction in basal flow would therefore appear to represent irreversible myocardial and resistive vessel injury.

The vasodilator response to dipyridamole was markedly reduced and improved to a small degree at 6 months. Two explanations may account for this. First, there may be a partial recovery in resistive vessel function in areas of viable myocardium within the infarct zone contributing to vasodilatation after dipyridamole but remaining a small percentage of overall vasodilator potential. This recovery may occur with resolution of interstitial oedema restricting microvascular luminal dimensions (Willerson et al, 1972) and of neutrophil plugging in post-ischaemic capillaries (Engler et al, 1983; Chatelain et al, 1987). The reflex increase in sympathetic activation after infarction may also acutely impair resistive vessel dilatation (Schwartz & Stone, 1977) (see below). Alternatively, any small improvement in the response to dipyridamole could simply reflect the higher mean arterial pressure, and thus, coronary perfusion pressure

at repeat scanning compared to the initial study. However, coronary resistances after dipyridamole at both scans were not significantly different in the infarcted region, making this a less likely explanation.

A relationship was seen between both myocardial blood flow at rest and after vasodilatation and the amount of water-perfusable tissue in the infarct region which is a measure of the viability of the residual tissue (Yamamoto et al, 1992; de Silva et al, 1992) (Chapter 3.9). This confirms an inter-relationship between myocyte cellular function and the integrity of the coronary microvascular bed after myocardial infarction. The longer delay there is to initiation of thrombolytic therapy and subsequent recanalisation, the greater the reduction in tissue viability. This finding is consistent with the benefit in survival established from large multi-centre trials of intravenous thrombolysis (ISIS-2, 1990).

An inverse relationship was demonstrated between basal myocardial blood flow in the remote region and that in the infarct region. Whether this reflects a regional steal phenomenon (so-called "reverse steal" from ischaemic to remote region), an intracardiac neural reflex, or altered regional intraventricular loading (Smalling et al, 1986) is unknown. In this respect, it has been demonstrated in an animal model that preconditioning of myocardium in one region by intermittent occlusion-reperfusion, may reduce infarct size when another artery in remote myocardium is subsequently occluded (Przyklenk et al, 1993). This so-called "remote preconditioning" occurred independent of collateral blood flow, haemodynamic parameters or area at risk. This could be further evidence for an increase in both α- and β-adrenergic sympathetic activation with catecholamine release mediating a preconditioning response, as described with neuronal noradrenaline release in the isolated rat heart (Locke-Winter et al, 1991). Alternatively, a protective factor such as adenosine may pass in collaterals or diffuse from capillary or post-capillary anastomoses to the region at risk. In this study, the inverse relationship between basal flow in infarct and remote myocardium

may be the first demonstration in man of "reverse steal". However, whether it is of importance in determining the extent of myocardial stunning or of outcome after infarction is unknown.

Myocardial Blood Flow in the Remote Region

The basal myocardial blood flow did not change significantly in the period between the two PET scans, and was similar to that measured in remote region of controls. At 8 days after infarction, the flow response to dipyridamole was significantly lower than that measured in the remote region of the control patients and did not correlate with the coronary vasodilator reserve in the infarct region, the enzymatic size of the infarction, or the time to thrombolysis. At the time of the second PET scan, the myocardial blood flow response, and thus the coronary vasodilator reserve was significantly greater, but it still remained significantly lower than in controls.

The reduced coronary vasodilator reserve in the remote region after infarction may be part of a generalized impairment of coronary vasodilatation observed by different groups using different methods in myocardial regions subtended by angiographically normal coronary arteries with disease elsewhere, compared with controls (Uren et al, 1993b; Sambuceti et al, 1991; Beanlands et al, 1992). In support of these findings, clinical and experimental studies have demonstrated that in the coronary circulation impaired endothelial-dependent dilatation to increased blood flow and to the endothelium-dependent vasodilator, acetylcholine, occurs before obstructive coronary artery disease develops (Cox et al, 1989; McLenachan et al, 1991; Sellke et al, 1990; Zeiher et al, 1991). This may be due to reduced production or release of nitric oxide (Sellke et al, 1990), as infusion of L-arginine, the precursor of nitric oxide, restores the impaired coronary blood flow response (Kuo et al, 1992). In an animal model of myocardial infarction, a reduced coronary vasodilator reserve has been shown in

remote myocardium due to both an increase in basal flow and to reduced vasodilatation, and this in part has been attributed to reactive myocyte hypertrophy in the non-infarcted myocardium (Karam et al, 1990). Furthermore, it has been shown that basal release of nitric oxide is impaired in post-infarction reactive hypertrophy several weeks after infarction (Drexler et al, 1992), perhaps due to functionally less active endothelium in the resistive vessels, as described after endothelial injury (Shimokawa et al, 1987). However, whether this mechanism accounts for the persistent reduction in the vasodilator response of the remote myocardium in this study is unknown.

In patients with acute myocardial infarction, these alterations may be accentuated by several mechanisms. First, following myocardial infarction there is a marked increase in sympathetic activity, which lasts for several days, associated with an increase in circulating catecholamine concentrations and activation of the renin-angiotensin-aldosterone system (McAlpine et al, 1988), the degree of which depends mainly on infarct size (McAlpine et al, 1988; Karlsberg et al, 1981). It is possible that the reduction in the coronary vasodilator reserve to dipyridamole in the remote regions could occur as a consequence of increased sympathetic activity following infarction (Minisi & Thames, 1991; Naccarella et al, 1984); in particular, increased α-adrenergic activity may cause a blunting of the vascular smooth muscle response to dipyridamole (Heusch, 1990), reducing the coronary vasodilator reserve at early scanning. Second, the coronary vasodilator reserve may have been affected by differences in blood pressure at the time of the two PET scans. The rate-pressure product was slightly but significantly lower at the first scan, because of a lower systolic blood pressure response to dipyridamole. When the resistive vessels are maximally dilated, the myocardial blood flow is proportional to the perfusion pressure providing the myocardial oxygen demand remains unchanged, ie. when blood pressure rises, myocardial blood flow will increase and the converse is true. However, this seems an

unlikely explanation for our findings since despite the small haemodynamic differences between the two scans, the coronary resistance in the remote regions after dipyridamole was significantly lower at repeat scanning, providing evidence of a change in resistive vessel function in the remote region in the period between the two scans. Third, following myocardial infarction, there is an acute increase in left ventricular diastolic pressure and, although this may fall over subsequent days to weeks (McKay et al, 1986), an acute rise in left ventricular diastolic pressures could impair the vasodilator response since most myocardial blood flow occurs during diastole (Domenech, 1978; Archie, 1978). Higher end-diastolic pressures would lead to an early increase in end-diastolic wall tension and a consequent increase in basal myocardial blood flow due to increased myocardial oxygen demand might be expected (Braunwald, 1971). A reduction in maximal flow due to an increase in resistance would reduce the maximal blood flow response to dipyridamole. However, we did not observe an increase in basal myocardial blood flow and in addition, transmural coronary flow is not thought to be affected significantly when coronary perfusion pressures are greater than 70 mmHg (Archie & Brown, 1974).

Conclusions

In patients after a mean of 8 days from a myocardial infarction, the coronary vasodilator reserve to dipyridamole is markedly impaired in the infarct region. Although unrelated to the residual severity of the infarct-related stenosis, a relationship exists between the vasodilator response to dipyridamole and the viability of the residual myocardium. To a lesser extent, the coronary vasodilator reserve is also impaired in the remote regions possibly due to acute resistive vessel dysfunction. The coronary vasodilator reserve in the remote myocardium undergoes an incomplete recovery after several months but still remains lower than in controls. Although the mechanism for both this acute and chronic alteration in resistive vessel function is

unknown, it may be of clinical relevance in the immediate and short-term management of patients after acute myocardial infarction.

CHAPTER 9. IMPAIRMENT OF THE MYOCARDIAL BLOOD FLOW RESPONSE TO COLD PRESSOR STRESS IN COLLATERAL-DEPENDENT MYOCARDIUM

9.1 Abstract

Myocardium subtended by an occluded epicardial coronary artery is dependent for perfusion on collateral vessels. The purpose of this study was to determine the effect of reflex sympathetic stimulation on coronary collateral and resistive vessel function. Eight patients, mean age 54±6 years (six male), with chronic stable angina were studied, in whom one coronary artery was occluded, but supplied by collaterals from the other angiographically normal arteries. All had normal left ventricular function, normal ECGs and positive exercise tests. All anti-anginal medication was discontinued for >48 h. Cold pressor after a mean duration of 5.9 min (range 5-6.5 min) caused chest pain in two, but none developed ECG changes. Regional myocardial blood flow (MBF) was measured in the collateral-dependent and in a remote region using positron emission tomography with i) ^{15}O-water at basal and at cold pressor, and ii) ^{18}F-fluoro-deoxyglucose (FDG). The coronary reserve was defined as cold pressor MBF/basal MBF. Heart rate was 62±7 beats·min^{-1} at basal and 67±12 beats·min^{-1} during cold pressor (p=NS). There was an increase in systolic blood pressure, 118±20 vs. 160±26 mmHg (p<0.01), and rate-pressure product, 7,420±1,820 vs. 10,800±3,080 mmHg·min^{-1} (p<0.01), at basal and during cold pressor, respectively. In a remote region, basal and cold pressor MBF were 1.03±0.25 ml·min^{-1}·g^{-1} and 1.55±0.57 ml·min^{-1}·g^{-1} (p<0.05 vs. basal MBF) respectively, and the coronary reserve was 1.51±0.45. In the collateral-dependent region, basal and cold pressor MBF were 0.93±0.21 ml·min^{-1}·g^{-1} (p=NS vs. remote

region) and 0.92±0.34 ml·min^{-1}·g^{-1} ($p<0.05$ vs. remote region) respectively, a coronary reserve of 1.01±0.44 ($p<0.05$ vs. remote region). In the remote region, with cold pressor, there was a significant decrease in the mean coronary vascular resistance from 97.8±32.6 mmHg·min·g·ml^{-1} to 81.5±31.9 mmHg·min·g·ml^{-1} ($p<0.05$) at basal and at cold pressor respectively, whereas in the collateral-dependent region, the mean coronary vascular resistance increased from 106.4±32.6 mmHg·min·g·ml^{-1} to 142.8±64.0 mmHg·min·g·ml^{-1} ($p<0.05$ vs. remote region). No increase in FDG uptake after cold pressor was seen in collateral-dependent regions compared to remote regions.

These data indicate that resistive vessel dilatation occurs with cold pressor in the remote region. In the collateral-dependent region, in response to the same stress, there is either a failure of the collateral or resistive vessels to dilate or an increased tendency to vasoconstrict, although this occurs in the absence of demonstrable transmural myocardial ischaemia.

9.2 Introduction

After coronary artery occlusion, there is often no myocardial necrosis in the jeopardised region because collateral coronary blood flow from other myocardial regions is sufficient to maintain basal perfusion and thus, myocardial viability (Frye et al, 1977; Schwartz et al, 1978). Early studies using thallium-201 imaging have also indicated normal myocardial perfusion in these collateral-dependent areas not only at rest but also on exertion (Rigo et al, 1979; Eng et al, 1982; Kolibash et al,1982). However, a more recent study using positron emission tomography has shown a reduced coronary vasodilator response to dipyridamole in collateral-dependent myocardium (McFalls et al, 1993). Furthermore, when cardiac work is increased by atrial pacing, regional wall motion abnormalities develop in the myocardial segments

perfused by collateral vessels (Schwartz et al, 1978), indicating a limitation of their inability to maintain adequate myocardial perfusion in the face of increased myocardial metabolic requirements. Pupita et al have suggested that in patients with coronary artery disease, changes in vasomotor tone at the level of the coronary collateral and resistive vessels may alter the ischaemic threshold (Pupita et al, 1990). Several other groups (Nabel et al, 1988; Mudge et al, 1976; Yeung et al, 1991) have suggested that this functional abnormality of the coronary collateral and resistive vessels which appears to occur occurring as part of the atherosclerotic process may lead to an altered sensitivity to sympathetic activation. This may be manifest by the collateral and resistive vessels showing either an inability to vasodilate adequately or an increased tendency to vasoconstrict inappropriately, and this might then result in the development of myocardial ischaemia. Myocardial blood flow in collateral-dependent myocardium may thus serve as a model of resistive vessel function (Gregg & Patterson, 1980).

Investigation of the vasomotor responses of the collateral and resistive vessels in man is problematic since it is difficult to exclude the effects of concomitant changes in the vasomotor tone of the epicardial coronary arteries. In order to avoid this problem as far as possible, we have investigated coronary blood flow in a group of patients with normal left ventricular function but who have one occluded coronary artery. Thus in these patients, one myocardial region is entirely dependent on collateral blood flow. In this study, cold pressor stress was used as a sympathetic nervous system stimulus. Regional myocardial blood flow and glucose metabolism was measured using positron emission tomography in order to compare the collateral-dependent region with that of a region subtended by an angiographically normal coronary artery.

9.3 Methods

9.3.1. Patient Population

Eight patients (mean age 54±6 years; 6 male) with single vessel coronary artery occlusion were studied. The occluded artery was the left anterior descending (LAD) in 2 patients, dominant left circumflex artery (LCX) in 2 patients, and dominant right coronary artery (RCA) in 4 patients. No patient had ECG evidence of previous myocardial infarction and all patients had normal left ventricular function (Table 9.1). All had a positive exercise test (>0.1 mV ST segment depression) at moderate workload on the modified Bruce protocol (Section 9.4, Table 9.2).

9.3.2. Study Protocol

The protocol was approved by the Research Ethics Committee of Hammersmith Hospital and all patients gave informed and written consent.

Patients were studied on three separate occasions. Any anti-anginal medications were discontinued at least 48 hours before any part of the study. On one occasion, exercise testing was performed under basal conditions and after sublingual nitrates. On another occasion, the other non-invasive stress tests were performed. The stress tests were i) mental arithmetic (serial 7 subtraction for up to 5 minutes), ii) handgrip (to 30% maximum grip for up to 5 minutes), and iii) cold pressor (immersion of right hand in ice for as long as tolerated, or up to 6 minutes). These stress tests were performed with continuous ECG monitoring, and a 12 lead ECG together with the blood pressure were recorded after each minute of the test. On the third occasion, patients underwent PET scanning at basal and during cold pressor.

9.3.2.1. Coronary Arteriography and Left Ventriculography

(Table 9.1)

All patients enrolled in the study had undergone coronary arteriography within 6

Table 9.1. Global and Regional Left Ventriculography

					Regional Wall Motion (% of contribution)				
Patients	Age/sex	Occluded Artery	Collateral Grade	LV Ejection Fraction (%)	Antero-basal (14.4-25.4)	Antero-lateral (10.1-17.3)	Apical (2.5-6.3)	Inferior (10.6-18.2)	Postero-basal (15.7-25.4)
1	56/M	RCA	G3 - LCX	82.3	20.3	16.7	4.2	15.8	25.2
2	63/F	RCA	G2 - LCX	70.9	16.5	12.2	4.9	18.1	19.2
3	56/M	LAD	G2 -LCX	81.5	21.3	18.0	4.8	17.8	19.4
4	43/F	RCA	G3 - LAD	86.4	23.5	17.5	4.5	16.5	24.4
			G3 - LCX						
5	48/M	RCA	G2 - LCX	75.8	21.0	17.1	5.2	15.8	16.7
			G3 - RCA						
6	51/M	LCX	G3 - RCA	64.9	18.1	12.9	4.2	14.2	15.5
7	56/M	LAD	G2 - RCA	76.5	20.5	15.6	4.0	13.6	22.8
			G2- LAD						
8	56/M	LCX	G3 - RCA	68.8	21.2	18.0	5.0	10.7	14.0
Mean±SD				75.8±7.5	20.3±2.1	14.7±4.0	4.6±0.4	15.3±2.4	19.5±4.2

The normal ranges of regional contribution to left ventricular function by the CREF method are given in brackets. Collaterals are graded accoridng to the method of Rentrop et al (see text) with the artery of origin of the collateral vessels. LV = left ventricular; LAD = left anterior descending artery, LCX = dominant left circumflex artery, RCA = dominant right coronary artery.

months of PET scanning. Coronary arteriograms were analysed by an automated edge contour detection computer analysis system (Chapter 5, Section 5.3.2.2) (Reiber et al, 1984). Collaterals were graded according to the method of Rentrop et al (Rentrop et al, 1985) (Chapter 7, Section 7.3).

Global and regional left ventricular function was measured according to the method described previously (Chapter 7, Section 7.3.2.1). In patients with an occluded LAD, the postero-basal and inferior regions were considered remote; in patients with a dominant LCX or RCA occlusion, the antero-basal and antero-lateral regions were considered remote from the site of coronary occlusion.

9.3.2.2. Measurement of Regional Myocardial Blood Flow and Metabolism

1. PET Scanning

Patients were fasted overnight and all had been off anti-anginal medication (except for sublingual nitrates) for at least 48 hours. No nitrates were taken for at least two hours before the study.

All PET scans were performed with an ECAT 931-08/12 camera (CTI Inc., Knoxville, Tennessee, USA) (Spinks et al, 1988). Regional myocardial blood flow (MBF, in $ml.min^{-1}.g^{-1}$) was measured in patients using oxygen-15-labelled water ($H_2{}^{15}O$) as a flow tracer using the previously validated $C^{15}O_2$ inhalation technique (Araujo et al, 1991). Measurements were made at basal and during the cold pressor stress. The protocol during cold pressor was as follows; after a background scan of 30 seconds, the right hand was immersed in ice simultaneous with the start of $C^{15}O_2$ inhalation, which was given for 210 seconds. The hand was removed from ice between the end of $C^{15}O_2$ inhalation and the end of the scan at 420 seconds. Systemic blood pressure and heart rate were monitored throughout the study and a 12 lead ECG was recorded every minute during the stress. Immediately following the cold pressor stress, 185 $MBq{\cdot}ml^{-1}$ of FDG was given by intravenous infusion over 5

minutes, and the duration of this dynamic scan was 65 minutes. These studies were done without glucose loading. Images were analysed over the last of 36 frames which had a duration of 5 minutes. Under ischaemic conditions, there is an increased utilization of glucose by the myocardium both from glycogen breakdown and from exogenous glucose (Chapter 3, Section 3.8). Global and regional changes in glucose utilisation under controlled conditions may be studied using FDG (MacGregor et al, 1977; Phelps et al, 1978; Bergmann et al, 1985), to demonstrate regions of myocardial ischaemia. In this study, regional FDG uptake was measured in absolute counts of activity (Bonow et al, 1991; Vanoverschelde et al, 1992).

The sinograms were corrected for attenuation and reconstructed on a Microvax II computer (Digital Equipment Corporation, Marlboro, Mass.,USA) (Spinks et al, 1988; Araujo et al, 1991). Images were transferred to a SUN 3/60 work-station for further analysis using Analyze™ (Mayo Foundation, Rochester, Minn., USA) and Pro-Matlab™ (The Mathworks Inc., South Natick, Mass., USA) software packages. Images were created and analysed as previously described (Araujo et al, 1991).

In patients with the LAD as the occluded artery, the anterior region was designated as the collateral-dependent region and the infero-posterior region as the remote region of interest. In patients with a dominant RCA or LCX as the occluded artery, the infero-posterior region was designated as the collateral-dependent region and the anterior region as the remote region of interest. Basal MBF and MBF during cold pressor were determined. The coronary reserve was defined as the quotient of cold pressor MBF to basal MBF.

To exclude the effect of changes in mean arterial pressure, and thus perfusion pressure, on coronary flow, regional coronary resistance was calculated from the quotient of mean arterial pressure and regional myocardial blood flow both under basal conditions and during cold pressor, and expressed as $mmHg \cdot min \cdot g \cdot ml^{-1}$.

With PET, the extent of myocardial damage may be determined from the background (transmission) scan generated by external positron irradiation of the patient, and the dynamic flow scan generated by $C^{15}O_2$ inhalation under basal conditions. As described in Chapter 3, the total anatomical tissue fraction (ATF, in $g \cdot ml^{-1}$ of region) is the perfused tissue fraction (PTF) and the non-perfused tissue fraction together. The ratio of PTF/ATF for each region may be determined in collateral-dependent and remote regions, and expressed as an index of water-perfusable tissue (PTI) (Iida et al, 1988). Previous work in our institution has shown a strong positive and negative predictive accuracy with the PTI for the recovery of myocardial contractility in hypokinetic or akinetic myocardial regions after myocardial infarction (Yamamoto et al, 1992), or after revascularisation (de Silva et al, 1992) (Chapter 3, Section 3.9.1).

9.3.2.3. Ambulatory Electrocardiographic Monitoring

Ambulatory ECG monitoring over a 24 period was performed with the patients off any anti-anginal medication for at least 48 hours in the case of calcium antagonists and 72 hours with β-blockers. Two-channel amplitude-modulated recorders (Marquette 8500) were used with the two chest leads showing the most impressive ECG changes at exercise testing selected. The effect on the ST segment of changes in posture was carefully assessed. Patients were provided with diaries to note the time at which episodes of angina occurred and the circumstances with which they occurred. Analysis was performed with a laser Holter system (Marquette 8000). An episode of transient ischaemia was defined as a horizontal or down-sloping depression of the ST segment of more than 0.1 mV occurring 80 msec after the J point and lasting longer than one minute. The duration of each ischaemic episode and the heart rate at the onset of the ST segment shift, in each of the preceding 10 minutes, and at 0.1 mV depression of the ST segment were measured.

Variability of the threshold of myocardial ischaemia was determined by two methods; i) >15% difference in heart rate at the onset of ST segment depression on exercise testing and ambulatory monitoring, and ii) >15% difference in minimum and maximum heart rate at the onset of ST segment depression.

9.3.3. Statistical Analysis

Results are expressed as mean±SD. Analysis of data was performed by the paired and unpaired Students t test where appropriate. A p value <0.05 was considered statistically significant.

9.4 Results

9.4.1. Exercise Testing

(Table 9.2)

Patients underwent exercise testing at basal and after sublingual nitrates. Rate-pressure product was 10,940±2,380 and 11,780±2,540 mmHg·min^{-1} at rest, and 25,470±6,050 and 26,020±5,810 mmHg·min^{-1} at peak exercise (both p=NS) under basal conditions and after nitrates, respectively (Table 9.2). All 8 patients had positive tests (≥0.1 mV ST segment depression) under basal conditions; after sublingual nitrates, one patient had a negative test and the other 7 increased their ischaemic threshold from a rate-pressure product of 19,650 to 23,095 mmHg·min^{-1} (Table 9.2), due to a increase in heart rate at the ischaemic threshold.

9.4.2. Non-Invasive Stress Testing

(Tables 9.3, 9.4 and 9.5)

Mental stress (Table 9.3), handgrip (Table 9.4) and cold pressor (Table 9.5) had no significant effect on heart rate, but all three increased systolic blood pressure and

Table 9.2. Haemodynamic Variables at Exercise Testing

	Basal State			0.1 mV ST Segment Depression			Peak Exercise		
Patient	Heart Rate (bts·min^{-1})	Systolic BP (mmHg)	Rate-Pressure Product (mmHg·min^{-1})	Heart Rate (bts·min^{-1})	Systolic BP (mmHg)	Rate-Pressure Product (mmHg·min^{-1})	Heart Rate (bts·min^{-1})	Systolic BP (mmHg)	Rate-Pressure Product (mmHg·min^{-1})
1	99	125	12,400	127	140	17,800	136	140	19,000
	110	140	15,400	153	135	20,500	153	135	20,500
2	83	140	11,600	110	160	17,600	145	180	26,100
	109	120	13,100	144	170	24,500	165	180	29,700
3	89	105	9,300	127	155	19,700	147	150	22,100
	105	110	11,600	162	138	22,400	162	138	22,400
4	78	115	9,000	115	165	19,000	173	195	33,700
	89	110	9,810	141	150	21,204	175	175	30,625
5	85	150	12,750	111	200	22,200	123	200	24,600
	96	150	14,400	142	220	31,240	150	200	30,000
6	83	140	11,600	148	178	26,340	161	185	29,780
	106	110	11,700	158	173	27,330	163	180	29,340
7	77	150	11,550	144	200	28,800	154	205	31,750
	77	140	10,780	-	-	-	151	200	30,200
8	62	110	6,820	108	138	14,900	118	142	16,750
	78	96	7,490	116	125	14,500	122	126	15,370
Mean±SD	82±11	129±18	10,940±2,380	121±14	162±22	19,650±3,690	145±19	175±27	25,470±6,050
	96±14†	122±19	11,780±2,540	145±15†	159±32	23,095±5,340*	155±15*	167±30*	26,020±5,810

* $p<0.05$, † $p<0.01$ vs. Basal.

thus the rate-pressure product. Mental stress caused only a small increase in mean arterial pressure compared to basal. However, isometric handgrip and cold pressor had marked effects on increasing both diastolic and mean arterial pressure. There were no significant ST segment changes (≥0.1 mV depression) with any of the non-invasive tests in any of the patients. There was no chest pain with mental stress or handgrip, but two patients reported mild chest pain during cold pressor.

9.4.3. PET Scanning

(Table 9.6; Figures 9.1 and 9.2)

Cold pressor testing was sustained for a mean duration of 5.9 min (range 5-6.5) at PET scanning. Cold pressor increased systolic blood pressure (118±20 to 160±26 mmHg, $p<0.01$) and rate-pressure product (7,420±1,820 to 10,800±3,080 $mmHg \cdot min^{-1}$, $p<0.01$) from basal to cold pressor respectively, but had no major effect on heart rate (62±7 to 67±12, p=NS). Two patients reported chest pain during cold pressor, but no significant ST segment changes were seen in any patient.

In the remote region, basal and cold pressor MBF were 1.03±0.25 and 1.55±0.57 $ml \cdot min^{-1} \cdot g^{-1}$ ($p<0.05$ vs. basal MBF) respectively, a coronary reserve of 1.51±0.45. In the collateral-dependent region, basal and cold pressor MBF were 0.93±0.21 (p=NS vs. remote region) and 0.92±0.34 $ml \cdot min^{-1} \cdot g^{-1}$ (p=NS vs. basal MBF, $p<0.05$ vs. remote region) respectively, a coronary reserve of 1.01±0.44 ($p<0.05$ vs. remote region) (Figure 9.1). In the remote region, coronary vascular resistance decreased from 97.8±32.6 to 81.5±31.9 $mmHg \cdot min \cdot g \cdot ml^{-1}$ ($p<0.05$) at basal and cold pressor, respectively. In the collateral-dependent region, coronary vascular resistance was 106.4±32.6 $mmHg \cdot min \cdot g \cdot ml^{-1}$ at basal (p=NS vs. remote region) (Figure 9.2). The coronary vascular resistance increased in 6 of the 8 patients with cold pressor, giving an overall mean value of 142.8±64.0 $mmHg \cdot min \cdot g \cdot ml^{-1}$. Although this was not significantly different from basal, it was higher than in the

Table 9.3. Haemodynamic Variables at Mental Stress

	Basal					Maximal Values				
Patient	Heart Rate (bts·min^{-1})	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Mean Arterial Pressure (mmHg)	Rate-Pressure Product (mmHg·min^{-1})	Heart Rate (bts·min^{-1})	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Mean Arterial Pressure (mmHg)	Rate-Pressure Product (mmHg·min^{-1})
1	80	140	100	113	11,200	85	160	100	120	13,600
2	89	120	85	97	10,680	91	130	78	95	11,830
3	85	110	82	91	9,350	87	112	82	92	9,744
4	76	120	80	93	9,120	78	130	85	100	10,140
5	66	150	100	117	9,900	67	160	100	120	10,720
6	65	110	70	83	7,150	75	120	80	93	9,000
7	80	160	90	113	12,800	79	175	90	118	13,825
8	58	110	70	83	6,380	62	115	72	86	7,130
Mean±SD	75±11	128±20	85±12	99±14	9,572±2,095	78±10	138±24†	86±10	103±14*	10,749±2,277†

* $p<0.05$, † $p<0.01$ vs. Basal.

Table 9.4. Haemodynamic Variables at Isometric Handgrip

	Basal					Maximal Values				
Patient	Heart Rate (bts·min^{-1})	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Mean Arterial Pressure (mmHg)	Rate-Pressure Product (mmHg·min^{-1})	Heart Rate (bts·min^{-1})	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Mean Arterial Pressure (mmHg)	Rate-Pressure Product (mmHg·min^{-1})
1	79	136	100	112	10,744	82	170	110	130	13,940
2	86	116	80	92	9,976	87	142	85	104	12,354
3	83	90	60	70	7,470	83	100	76	84	8,300
4	64	125	85	98	8,000	75	140	85	103	10,500
5	64	155	105	122	9,920	83	200	120	147	16,600
6	61	120	85	97	7,320	70	150	95	113	10,500
7	71	140	90	107	9,940	67	180	125	143	12,060
8	59	114	70	85	6,726	58	126	80	95	7,308
Mean±SD	71±11	124±20	84±15	98±16	7,637±3,077	76±10	151±32†	97±19*	115±23†	11,445±2,995*

* $p<0.05$, † $p<0.01$ vs. Basal.

Table 9.5. Haemodynamic Variables at Cold Pressor

	Basal					Maximal Values				
Patient	Heart Rate (bts·min^{-1})	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Mean Arterial Pressure (mmHg)	Rate-Pressure Product (mmHg·min^{-1})	Heart Rate (bts·min^{-1})	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Mean Arterial Pressure (mmHg)	Rate-Pressure Product (mmHg·min^{-1})
1	82	110	80	90	9,020	79	140	105	117	11,060
2	80	115	80	92	9,200	84	178	90	109	14,952
3	75	90	65	73	6,750	76	100	80	87	7,600
4	68	120	90	100	8,160	76	125	90	102	9,500
5	66	155	100	118	10,230	65	210	120	150	13,650
6	68	120	80	93	8,160	65	150	100	117	9,750
7	69	145	95	112	10,005	72	165	105	125	11,880
8	52	96	70	79	4,490	51	144	80	101	7,340
Mean±SD	70±9	119±22	82±12	95±15	8,252±1,886	76±10	151±32†	96±14†	114±19†	11,445±2,995†

* $p<0.05$, † $p<0.01$ vs. Basal.

Table 9.6. ^{18}F-Fluoro-deoxyglucose Uptake and Myocardial Viability

	FDG Uptake		Perfusable Tissue Index	
Patients	Collateral-dependent region	Remote region	Collateral-dependent region	Remote region
1	2,381±342	2,605±146	0.86	1.04
2	651±223	676±78	0.85	1.02
3	583±92	668±65	1.09	1.18
4	598±75	653±41	0.86	1.00
5	1,187±123	1,108±86	0.83	1.12
6	1,345±276	1,401±139	0.79	0.85
7	761±142	846±105	0.88	0.93
8	3,382±586	3,336±238	0.76	0.86
Mean±SD	1,363±1,096	1,413±1,096	0.86±0.10 **	1.00±0.12

** $p<0.01$ vs. remote region. FDG uptake is measured in counts·pixel^{-1}.

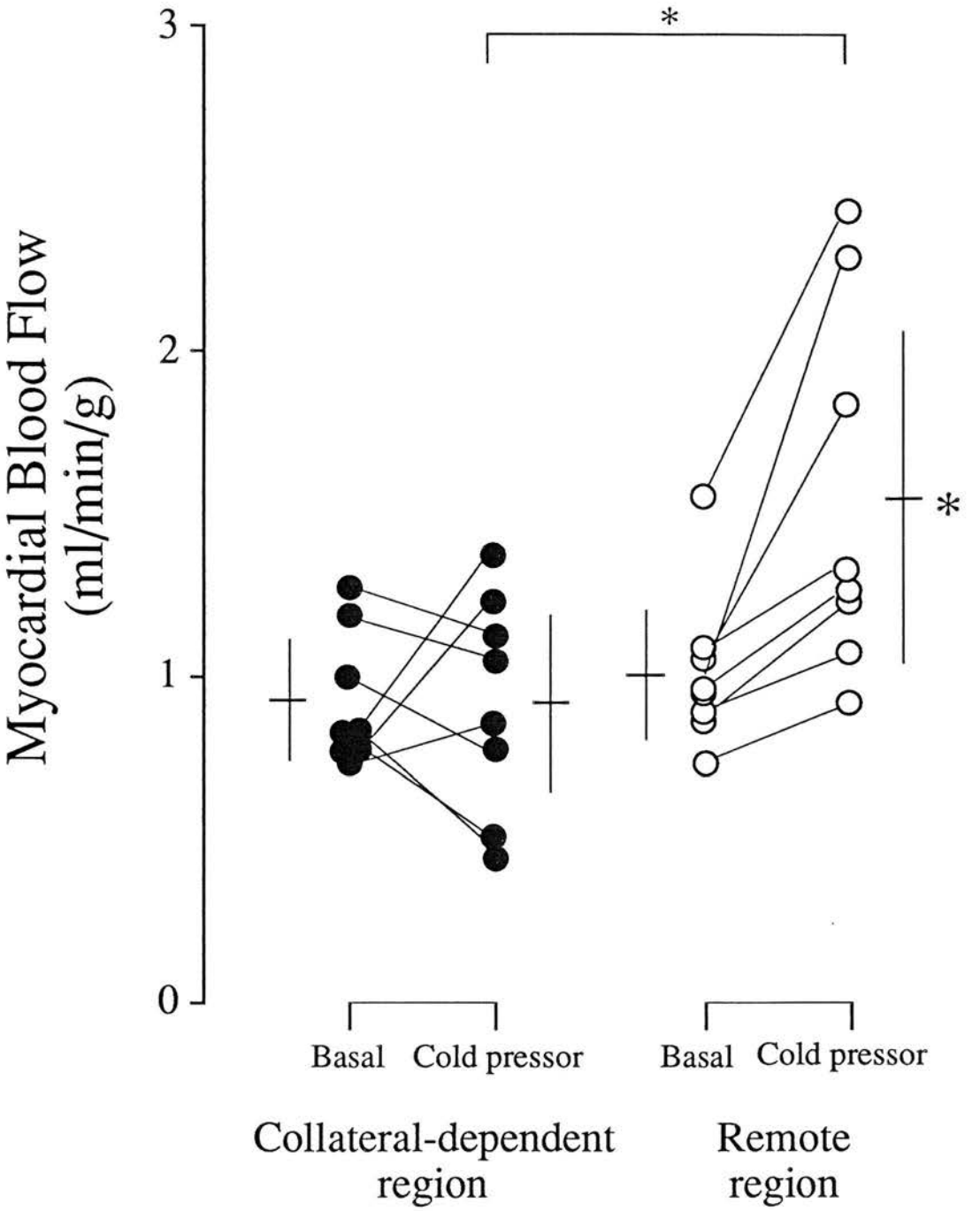


Figure 9.1. Individual values of the myocardial blood flow response from basal to cold pressor in collateral-dependent (black symbols) and remote regions (open symbols). * $p<0.05$ vs. Basal.

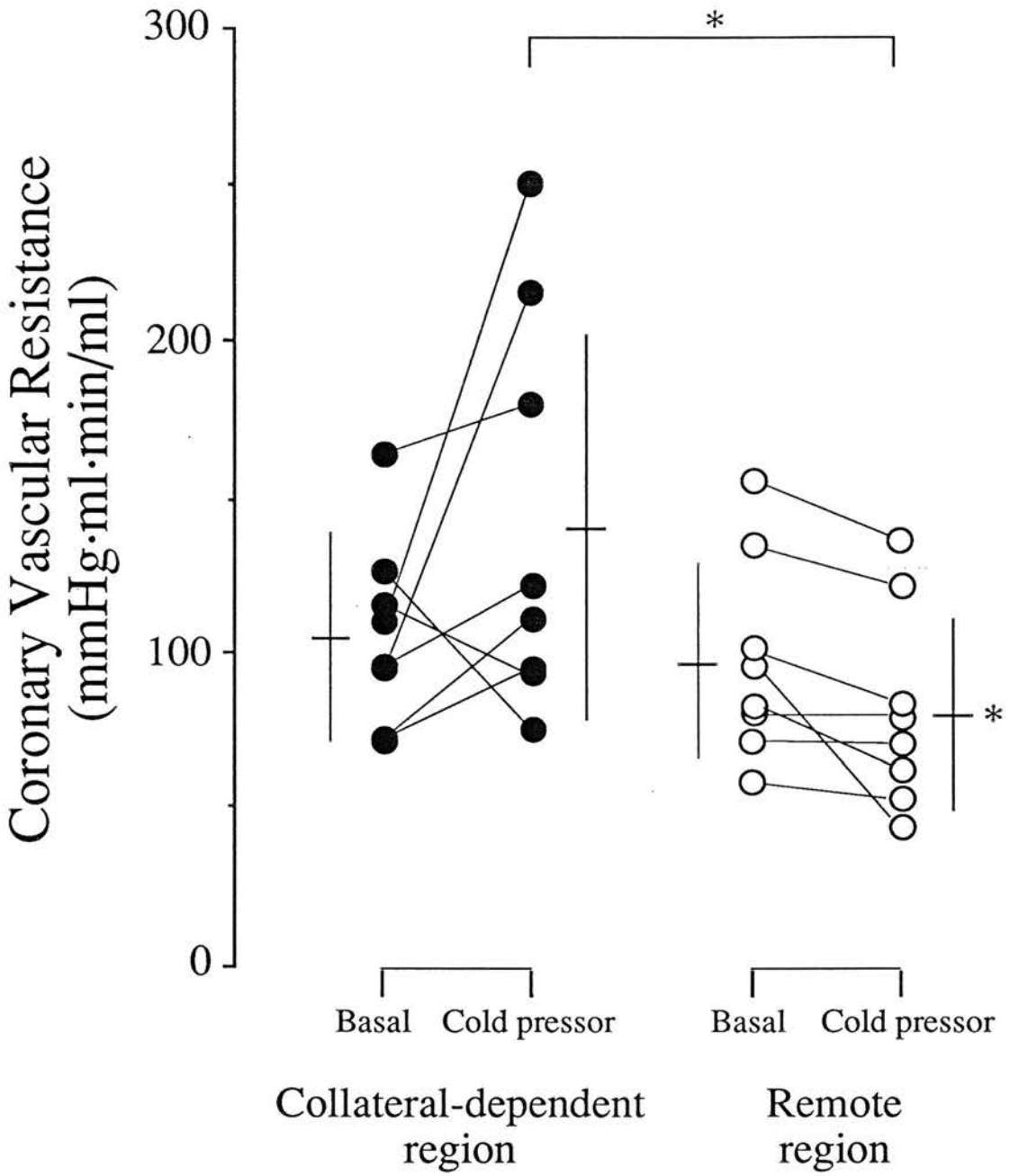


Figure 9.2. Individual values of the coronary resistance at basal and cold pressor in collateral-dependent (black symbols) and remote regions (open symbols). * $p<0.05$ vs. Basal.

remote region ($p<0.05$) (Figure 9.2).

No regional increase in FDG uptake was seen in collateral-dependent regions compared to remote regions, after cold pressor (Table 9.6). The PTI, although higher in remote regions, was greater than the threshold of 0.7 in the collateral-dependent regions (Table 9.6). No correlation was demonstrated between PTI and basal MBF, cold pressor MBF, or the coronary blood flow response in the collateral-dependent region.

9.4.4. Ambulatory ECG Monitoring

(Table 9.7; Figure 9.3)

Four patients had transient episodes of ≥0.1 mV ST segment depression and four had no episodes within a 24 hour period (Table 9.7). All 4 patients with ST segment depression had evidence of variable threshold myocardial ischaemia, using the definition of the difference between heart rate at the onset of ischaemia on exercise and on Holter, although only two demonstrated variability when comparing episodes of ischaemia on Holter alone (Section 9.3) (Table 9.7). Comparing these two subgroups with and without ambulatory myocardial ischaemia, the 4 patients with recurrent ambulatory ischaemia had a higher basal MBF in the collateral-dependent region (1.08 ± 0.20 vs. 0.79 ± 0.05 $ml \cdot min^{-1} \cdot g^{-1}$, $p<0.05$), although the MBF response to cold pressor (0.86 ± 0.29 vs. 0.97 ± 0.42 $ml \cdot min^{-1} \cdot g^{-1}$, p=NS) and ultimately coronary reserve (0.78 ± 0.14 and 1.24 ± 0.54, p=NS) in those with and without ambulatory ischaemia, was not significantly different (Figure 9.3). It remains likely that the lack of a difference in coronary reserve reflects the small numbers in the study.

Table 9.7. 24 Hour Ambulatory Electrocardiographic Monitoring

Patient No.	Pain	Duration (minutes)	HR at 0.1 mV ST Depression on Holter	HR at 0.1 mV ST Depression on Exercise	Difference in HR between Exercise Test and Holter	Difference Between Min. and Max. Holter HR
2	-	33	102	110	8	0
	-	12	108		2	6
	-	15	104		6	2
	-	5	109		1	7
	-	15	144		-34	42
3	+	10	103	127	24	11
	+	8	100		27	8
	+	6	105		22	13
	+	10	92		35	0
	+	40	94		33	2
	+	35	100		37	8
	+	15	144		-17	52
6	-	7	137	148	11	11
	-	6	126		22	0
	-	12	132		16	6
7	+	26	107	144	37	4
	+	15	105		39	2
	+	10	108		36	5
	+	15	103		41	0
	+	10	109		35	6
	+	15	105		39	2

HR = heart rate (beats·min^{-1}). Patients 1, 4, 5 and 8 had no episodes of significant ST segment depression during daily activity. (+) or (-) denotes painful or painless episodes of myocardial ischaemia. Duration refers to the time period from the beginning to the end of an episode resulting in ≥0.1 mV ST segment depression. Heart rates at the onset of 0.1 mV ST segment depression either on exercise at Holter monitoring are given. Differences described are between exercise and Holter heart rates at the beginning of ischaemia, and between the minimum and maximum heart rate recorded at the beginning of ischaemia on Holter.

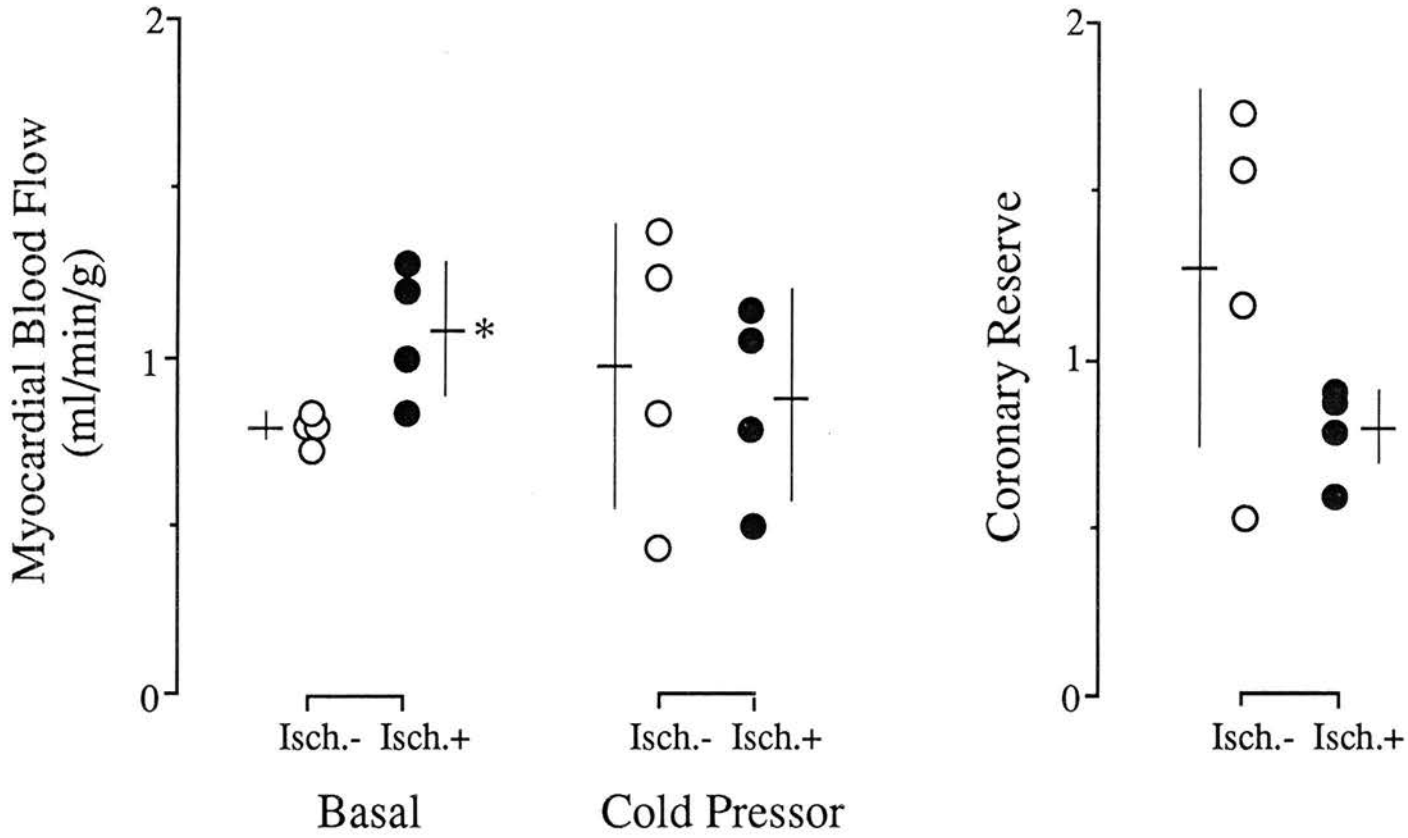


Figure 9.3. Individual values for myocardial blood flow in collateral-dependent myocardium under basal conditions and at cold pressor, and the coronary reserve, in patients subdivided into those with (isch.+) and without (isch.-) transient myocardial ischaemia on ambulatory ECG monitoring. * $p<0.05$ vs. Isch.-.

9.5 Discussion

In this study, mental arithmetic, handgrip and cold pressor testing caused no demonstrable myocardial ischaemia, as measured by significant ST segment change on continuous ECG, despite an increase in myocardial oxygen demand, measured as the rate-pressure product. However, the peak rate-pressure product achieved with these non-invasive stress tests was much lower than the ischaemic threshold determined at treadmill exercise testing, making significant myocardial ischaemia unlikely without an additional (primary) reduction in coronary flow (Maseri, 1983). Four of the patients did demonstrate variable threshold myocardial ischaemia, which although lower than the heart rate at the ischaemic threshold of myocardial ischaemia on exercise, was still higher than that during any of the non-invasive stress tests. This may be an indication of an additional vasoconstrictive component in the genesis of ischaemia in these patients (Pupita et al, 1990). It is also of interest that patients with transient ischaemia at ambulatory monitoring had higher basal flow, perhaps suggesting early impairment in the autoregulation of myocardial perfusion (Chapter 1, Section 1.4).

Cold pressor, an α-adrenergic stimulus (Mudge et al, 1976), did however induce chest pain in two patients. There was no difference between the basal myocardial blood flow in the collateral-dependent myocardium and the remote region at PET, indicating that the collateral vessels were sufficient to maintain resting perfusion. Reflex sympathetic stimulation using cold pressor did result in an increase in myocardial blood flow in remote myocardium, but not in viable, collateral-dependent myocardium, with a value much lower than that in the remote region. Indeed, in 6 of the patients, cold pressor resulted in an increase in coronary resistance, although there was no overall difference in regional FDG uptake in any of the 8 patients, excluding prolonged transmural myocardial ischaemia as a consequence of this inappropriate resistive vessel vasoconstriction or inability to vasodilate.

Coronary Collaterals and Myocardial Perfusion

In the dog model, a coronary stenosis does not stimulate collateral development until the stenosis decreases luminal area by about 80%, but this collateral growth is much less than that which occurs in response to compete coronary occlusion (Schaper et al, 1988). The vasomotor function of collaterals remains poorly understood. Although, in animal models, the functional capacity of collateral vessels is unrelated to visual assessment, in clinical studies, patients with larger collaterals generally have a higher peripheral coronary perfusion pressure and retrograde flow into diseased vessels (Goldstein et al, 1974; 1975). Under normal conditions, in a dog model, the resistance through native collateral vessels is much higher, up to 60-80 times greater than the minimal resistance through the coronary resistive vessels (Schaper et al, 1987). However, using microspheres, maximal flow to an ischaemic zone via native collaterals may approach 30-50% of normal flow implying resistance only 2-3 times normal in the beating dog heart (Becker & Pitt, 1971; Marcus et al, 1975). Much of this variability occurs because of the problem of heterogeneous tissue sampling from both normal and collateral dependent areas (Schaper et al, 1976). Positron emission tomography may be used to measure regional blood flow without such limitations, and has shown that there is a functional impairment of collateral vessels when supplying a myocardial territory, without support from other epicardial vessels (McFalls et al, 1993).

Collateral Vasomotion and Resistive Vessel Function

There is some evidence which tends to suggest that α_1-adrenergic mechanisms are unlikely to modulate collateral vasomotion (Harrison et al, 1986). In one study, noradrenaline (combined α_1- and α_2-adrenergic stimulation) did not alter trans-collateral resistance in an open-chest dog model, although of interest selective α_2-adrenergic stimulation alone with the agonist BHT 920 did increase trans-collateral

resistance (Maruoka et al, 1987), perhaps implying an α_2-adrenergic component to the modulation of collateral vasomotion. In contrast, mature collaterals have functional β-adrenergic receptors to a similar extent to other coronary vessels; increased β-adrenergic stimulation leads to vasodilatation thus improving collateral perfusion at times of stress (Harrison et al, 1991).

Only coronary collaterals greater than 100-200 μm are apparent angiographically, and are responsible for at least 50% of the coronary resistance (Marcus et al, 1990). As smaller resistive vessels, the arterioles, have no neural efferents, it is possible that the impaired response to the predominately α-adrenergic stimulation of the cold pressor test reflects an increased tendency to vasoconstriction in the resistive vessels greater than 100 μm, the so-called "pre-arterioles" (Maseri et al, 1991) (Chapter 1, Section 1.4.1.4). Unlike the arterioles which are directly influenced by cellular metabolism and tissue pH, these pre-arterioles respond to changes in coronary blood flow and thus perfusion pressure, to ensure the match of flow to metabolic requirements. It is possible that in coronary artery disease, abnormal resistive vessel function in the pre-arterioles may cause or contribute to myocardial ischaemia, if there is sufficient impairment of vasomotor function in these vessels with an increase in myocardial demand. Previous work has shown that infusion of noradrenaline (Chilian et al, 1989b), and serotonin (Lamping & Eastham, 1989), constrict resistive vessels greater than 100 μm, but dilate smaller vessels. It is possible therefore that the local vasoconstrictor response in the collateral-dependent region, is an inappropriate response to α-adrenergic stimulation by the resistive vessels perfused by the collaterals (Heusch, 1990), occurring with the reflex α-adrenergic stimulation of the cold pressor test. However, whether this inappropriate vasoconstriction is ever sufficient to cause myocardial ischaemia in its own right, is not known.

An additional mechanism for impaired resistive vessel dilatation or inappropriate vasoconstriction is endothelial dysfunction in chronically perfused mature collaterals.

This has been suggested by the vasoconstrictor response of collateral-dependent myocardium in open chest dogs to physiological doses of vasopressin (Peters et al, 1989). This has been confirmed using in vitro microvessel imaging; relaxation to the endothelium-dependent vasodilators acetylcholine and ADP are markedly impaired in resistive vessels from collateral-dependent myocardium compared to controls (Sellke et al, 1990). Two possibilities for this impairment are chronic hypoperfusion down-regulating nitric oxide synthetase from membrane receptors or growth factors from collateral vessels causing proliferation of functionally less active resistive vessel endothelium (Harrison et al, 1991) (Chapter 1, Section 1.4.3).

The decrease in myocardial blood flow in the collateral-dependent myocardium with cold pressor could also occur due to a collateral steal effect ie. a relative reduction in collateral flow due to an increase in flow to normal myocardium. This has been demonstrated in intact animals (Cohen et al, 1976), although often confounded by the effect of pharmacological vasodilatation on systemic haemodynamics. In a study using an isolated heart, during adenosine vasodilatation, collateral perfusion appeared to be inversely related to perfusion in normal myocardium, a five-fold increase in flow to normal myocardium associated with a 50% reduction in collateral flow (Simonetti et al, 1987). This reverse steal may of course still occur because of an inability of resistive vessels to vasodilate adequately in the collateral-dependent myocardium.

Conclusions

There is impaired resistive vessel function in myocardial regions entirely perfused by collateral vessels, with normal basal myocardial blood flow and contractile function at rest. Overall, there is an inability of the collateral or resistive vessels to vasodilate adequately or an inappropriate vasoconstrictor response to cold pressor compared to remote myocardium. This abnormality occurred at a cardiac workload lower than that associated with exercise-induced myocardial ischaemia, and this model of resistive

vessel dysfunction was unassociated with objective evidence of transmural myocardial ischaemia. Nonetheless, this phenomenon may still contribute to causing ischaemia perhaps in the presence of an increased sympathetic drive or a reduction in coronary perfusion pressure.

CHAPTER 10. SUMMARY AND PERSPECTIVE

10.1 The Evidence for Resistive Vessel Dysfunction in Clinical Models of Coronary Artery Disease

The objective of this thesis has been to describe coronary resistive vessel function which has been suggested as a cause of an impaired coronary vasodilator reserve by several independent groups in the past (Wilson et al, 1988; Pupita et al, 1990; Marcus et al, 1990). Although a resistive vessel abnormality has been suggested as a cause of reduced vasodilator responsiveness in other conditions such as hypertension, after cardiac transplantation and in syndrome X (Chapter 1), the prevalence in coronary artery disease is poorly described and understood. However, in several models described in this thesis, coronary resistive vessel dysfunction has been defined and the time course of the abnormality carefully described. Although the mechanism(s) for these abnormalities remain poorly understood, several lines of research have been suggested on the basis of the findings reported here. A hypothesis for the development of coronary resistive vessel dysfunction at the level of the pre-arteriolar vessels has been suggested based on existing experimental studies. It is now imperative that the clinical relevance of coronary resistive vessel dysfunction and the mechanism of dysfunction at the level of the pre-arteriolar vessels is defined.

Following the removal of a significant epicardial coronary artery stenosis by successful coronary angioplasty, the coronary vasodilator reserve is unchanged from pre-angioplasty values. For the first time, the components of this reduction have been defined and described in Chapter 5. The vasodilator response to dipyridamole is impaired acutely and persists for at least 24 hours. It is likely that this is due to the angioplasty procedure itself perhaps due to platelet activation, endothelial disruption and vasoconstrictor release, and/or embolisation of debris, as the recovery is relatively

quick. Basal myocardial blood flow takes over a week to recover which suggests that there has been a chronic adaptation to the reduction in coronary perfusion pressure. There is evidence suggesting a process of vascular remodelling in the coronary microcirculation distal to a severe epicardial stenosis, and this could account for the longer time period for the recovery of basal flow to lower normal values. In the absence of restenosis, the recovery of resistive vessel function is sustained at three months follow-up and the coronary vasodilator reserve is the same as described in remote regions of patients with stable single vessel disease.

The mechanism for this acute abnormality in coronary resistive vessel function after coronary angioplasty was investigated in Chapter 6. Several groups have proposed that coronary vasodilatation at a microvascular level is impaired as part of the atherosclerotic process and due to impaired production or release of EDRF (nitric oxide) (Drexler et al, 1991: Kuo et al, 1992). As previous experimental work has suggested that the vascular smooth muscle response to exogenous nitric oxide donors is enhanced by impaired endothelial function, this hypothesis was tested with regard to the impaired coronary vasodilator reserve after angioplasty. However, intracoronary sodium nitroprusside at a dose sufficient to cause systemic effects did not augment the impaired coronary vasodilator response to dipyridamole. Thus, the acute impairment of the coronary vasodilator reserve is probably not due to reduced production or release of nitric oxide at the level of the resistive vessels, but does not exclude an impairment of release or production of other endothelium-derived vasodilator products. Alternatively, this finding may be further evidence for injury at the level of the epicardial vessel wall during angioplasty balloon inflation as a factor in the impaired vasodilator response.

It has been assumed that when studying the coronary vasodilator reserve in one region of myocardium in patients with coronary artery disease that remote myocardium subtended by an angiographically normal artery is functionally normal. However,

given evidence for both impaired epicardial artery dilatation and an impaired coronary vasodilator response in hypercholesterolaemia (Drexler et al, 1991; Zeiher et al, 1991) and early atherosclerosis (Cox et al, 1989; Sellke et al, 1990; McLenachan et al, 1991), it is likely that resistive vessel dysfunction is present before the angiographic development of disease. This was demonstrated by comparing myocardial blood flow the remote regions of patients with single vessel coronary artery disease with a group of normal controls (Chapter 7). The coronary vasodilator reserve, although higher than that in the stenosis-related region, was lower than that in the controls. This was due to both a reduction in the vasodilator response to dipyridamole as well as a higher basal flow. The mechanisms of abnormality in basal and vasodilator flow are unclear. There is no previous evidence to suggest that the atherosclerotic process leads to an alteration in basal flow itself. A possible mechanism for this could therefore be an increase in sympathetic nervous activity resetting the autoregulation of basal flow at the level of pre-arteriolar vessels. Additionally, although atherosclerosis may diminish maximal resistive vessel dilatation, increased α-adrenergic stimulation in the coronary microcirculation may also cause this reduction. Measurement of metabolic substrate handling showed no difference between remote myocardium and normal controls in the basal state however. During pacing, increased glucose and alanine uptake in the absence of net lactate production did occur in remote regions compared to controls, which may be evidence of increased regional sympathetic activity. This functional difference independent of systemic haemodynamics but in the presence of regional ischaemia and wall motion abnormality elsewhere may be an early marker of the functional consequences of resistive vessel dysfunction.

Further evidence for the interaction of the sympathetic nervous system and the atherosclerotic process was demonstrated in patients after myocardial infarction (Chapter 8). In the infarct region, there was a marked reduction in the coronary vasodilator reserve consistent with acute myocardial injury, with a small degree of

recovery at follow-up several months after the event. Basal flow in the infarct region and the remote region were inversely related to each other perhaps indicating an inter-relationship between different myocardial regions such as "reverse steal" of myocardial blood flow away from areas of ischaemia. In the remote region in the acute phase of recovery, there was a marked impairment of the vasodilator response to dipyridamole which made only a partial recovery at subsequent follow-up compared to remote regions in stable patients with uncomplicated myocardial infarction. This may be evidence for an acute resistive vessel dysfunction remote from and independent of both coronary occlusion and reperfusion injury pertaining to the infarct region. Although the neurohumoral response to myocardial infarction involves an increase in α-adrenergic sympathetic activity which could impair vasodilator responsiveness acutely, it is equally possible that vasoconstrictor agents such as endothelin released in increasing concentrations after myocardial endothelial injury could impede the vasodilator reserve.

The relationship between resistive vessel function and myocardial viability was also examined in Chapter 8. Residual basal flow and the ability of coronary resistive vessels to vasodilate following direct injury appears to be closely related to the fraction of perfused tissue in the same myocardial region of interest. This measure of myocardial viability also correlates with the time to thrombolysis, consistent with other clinical studies. This is the first evidence to suggest that there may be a relationship between "microvascular stunning" and myocardial stunning (Bolli, 1992). Whether the integrity of a proportion of resistive vessels ie. a threshold of vasodilatation, is a determinant of recovery of myocardial contraction or simply a reflection of the amount of viable muscle and thus viable microvascular bed is not known.

Visible coronary collateral vessels derived from an angiographically normal epicardial artery sufficient to maintain normal contraction and basal myocardial blood flow were used as final model of resistive vessel function (Chapter 9). Using cold

pressor, a means of eliciting reflex α-adrenergic sympathetic stimulation, it was shown that collateral vessels are unable to permit any significant increase in myocardial blood flow to the collateral-dependent myocardium. In the majority, it was possible to demonstrate an inappropriate vasoconstrictor response to cold pressor compared to remote myocardium. Although again this could be interpreted as increased sensitivity to α-adrenergic sympathetic stimulation in the pre-arteriolar vessels, it was unassociated with objective macroscopic evidence of myocardial ischaemia.

These models are to a large part observational and it has proven difficult to demonstrate a cause and effect relationship between resistive vessel dysfunction and objective evidence of myocardial ischaemia. The next stage of investigation should be to determine the mechanism of resistive vessel dysfunction using other methodologies and to determine therapeutic strategies to counter or reverse this early but potentially devastating abnormality of coronary blood flow.

10.2 Future Studies

The work reported in this thesis has shown that coronary resistive vessel dysfunction occurs in several different coronary artery disease syndromes; both chronic and acute. Many questions regarding the mechanism, site and clinical significance of this abnormality still need to be addressed; a combination of clinical investigation together with experimental models will be necessary to define further the mechanisms controlling the behaviour of the coronary microvascular bed and the control of myocardial perfusion. I think that several areas of research could be usefully pursued and these are described below:

1. The mechanism of resistive vessel dysfunction after angioplasty will become more apparent with detailed study of platelet activation during angioplasty and the development of techniques to image or quantify endothelial and vascular smooth muscle function in vivo. Further studies are required to determine the correlates of an impaired coronary vasodilator reserve with respect to disease history, total ischaemic burden before angioplasty, and microvascular remodelling. Larger studies should be performed to examine whether there is any relationship between resistive vessel dysfunction and the process of coronary restenosis.

2. The influence of endothelial cell integrity on resistive vessel function is controversial. Most experimental work on the role of endothelium has been confined to the study of epicardial vessels which have little influence on coronary blood flow in unobstructed vessels. There is evidence to suggest that nitric oxide is not the only endothelium-dependent vasoactive influence on the control of microvascular flow, as inhibition of nitric oxide production has no effect on the vasodilator action of acetylcholine at a microvascular level (Lefroy et al, 1993). Furthermore, it is unclear at which level EDRF operates in the microvascular bed, although the hypothesis suggested at the beginning of the thesis would favour a role at the level of the pre-arteriolar resistive vessels. Studies of nitrovasodilators in experimental models of the human microvasculature would confirm this hypothesis. Studies using the precursor of nitric oxide, such as L-arginine, given at the time of coronary angioplasty may help to define the role of the endothelium in determining resistive vessel function after the procedure. The role of the various plasma lipid components and microvascular dysfunction requires further study.

3. The role of the sympathetic nervous system in the development and modulation of resistive vessel dysfunction should be elucidated. Many methods now exist to

quantify the effect of the autonomic nervous system on the cardiovascular system such as spectral analysis of heart rate variability (Malliani et al, 1991), and QT interval dispersion (Day et al, 1991), as well as more traditional methods of autonomic function testing and cathecholamine measurement. PET may now be used to measure the end-effects of sympathetic nervous system activity with the in vivo quantitation of β-receptor density in myocardium (Merlet et al, 1993) and pre-junctional reuptake-1 receptor density (Schwaiger et al, 1991). Techniques should be developed to study the direct effect of adrenergic afferent activity on resistive vessel function and its modulation by specific therapy, with particular reference to the α-adrenergic component of the sympathetic system. Since acute resistive vessel dysfunction occurs in remote myocardium after myocardial infarction, a particular line of investigation may include the mechanism whereby β-blockade reduces mortality after infarction, and whether its effect is predominately on remote myocardium.

4. The relationship between resistive vessel dysfunction and myocardial viability should be investigated further. Since hibernating myocardium is defined by a reduction in contractility and myocardial oxygen demand together with basal myocardial blood flow (Braunwald & Rutherford, 1986), it would be of interest to determine the magnitude of the reduction in the coronary vasodilator reserve that occurs in association, as an understanding of changes at the level of the resistive vessels may help to elucidate the mechanism of hibernation. Whether or not the resistive vessel function is further affected by myocardial stunning and determines recovery of contractility independent of or dependent on the amount of viable tissue should also be established.

5. The clinical significance of resistive vessel dysfunction requires further validation. This requires correlative study of PET scanning with other techniques used to

document myocardial ischaemia. Ischaemia caused by altered resistive vessel function may be predominately subendocardial and not accurately quantified by techniques dependent on ST segment depression such as exercise treadmill testing or Holter monitoring. Even using a well-validated non-invasive technique such as FDG scanning may not be adequately sensitive to detect transient non-transmural ischaemia. With the development of other tracers such as ^{11}C-acetate and $^{15}O_2$ to measure oxidative metabolism and an improvement in the resolution of PET cameras, this may become possible. Other techniques such as echocardiography and magnetic resonance spectroscopy may complement developments in the measurement of ischaemia with PET. Nonetheless, in order to extend the observation of resistive vessel dysfunction to a wider arena, it is imperative that other methods of demonstrating this important abnormality are developed in addition to invasive Doppler catheterisation and non-invasive positron emission tomography.

10.3 Conclusions

Coronary artery disease is the major epidemic afflicting the developed world in this latter half of the twentieth century, accounting for two-thirds of all premature deaths. Much clinical and experimental research has focussed on the effect of atherosclerosis on epicardial coronary arteries and the territory subtended by the diseased vessel. In this thesis, I have tried to show that the coronary resistive vessels which are to a large part invisible may cause or contribute to causing some of the pathophysiological manifestations of the coronary artery disease process. Furthermore, as a consequence, I have shown that coronary resistive vessel dysfunction occurs not only in the territory subtended by a diseased epicardial vessel but also in myocardium remote from the visible atherosclerotic process, in both the acute and chronic state. It is only with wider acceptance of coronary resistive vessel

dysfunction into modern clinical practice that its true relevance will be determined. Then, with wider confirmation of these findings, it will cease to be simply an interesting laboratory phenomenon or clinical anomaly and may be seen as one of the major influences on the outcome of acute and chronic coronary artery disease states.

APPENDIX 1.

The Application of ^{15}O-Water Delivery to the Measurement of Myocardial Blood Flow

The behaviour of ^{15}O-water in tissue can be described by the following equation for a single tissue compartment model:

$$dC_t(t)/dt = F.C_a(t)-(F/V_d+l).C_t(t) \quad (1)$$

where $C_t(t)$ is the regional tissue concentration of $H_2{}^{15}O$, $C_a(t)$ the arterial whole blood concentration of $H_2{}^{15}O$, F the regional flow in $ml \cdot min^{-1} \cdot ml^{-1}$, V_d the volume of distribution of water, and l the decay constant of ^{15}O.

The solution to this differential equation is as follows:

$$C_t(t) = F.C_a(t) * \exp(-[F/V_d + l].t) \quad (2)$$

where * denotes the operation of convolution. $C_t(t)$ represents the tissue response to arterial input function $C_a(t)$.

In Equation 1, two assumptions are made: i) both F and V_d are constant during the measurement period, ii) water is freely diffusible (ie. the extraction fraction of water is unity and no binding of water in tissue occurs). In practice, it is the actual concentration in within a region of interest (which includes blood) which is measured rather than the actual tissue concentration. However, it is assumed that the venous and tissue concentrations may be treated as a single compartment for freely diffusible tracers such as $H_2{}^{15}O$. Furthermore, the arterial activity is negligible because of its small volume. Thus Equations (1) and (2) hold with these assumptions.

Compared to the brain, there are no diffusion limitations in the heart because of the much larger permeability surface area. Thus one may calculate from the estimated value for permeability and surface area (c. 1,000$cm^2 \cdot ml^{-1}$) that even for flows as high as 8 $ml \cdot min^{-1} \cdot g^{-1}$ there will be an extraction fraction for water of at least 98%. For a freely diffusible tracer, venous and tissue concentrations may be considered similar if the volume of distribution of the tracer in tissue is unity, which is virtually so.

The main problem for the application of Equations (1) and (2) to myocardium is the invalidity of the assumption that the arterial fraction of blood vessels is low. More important than this consideration is the contamination of signal by spillover from the cardiac chambers, which may be higher than that from arterial blood. Thus, Equation (2) may be expanded to allow for spillover:

$$C_{tot}(t) = C_t(t) + V_a.C_a(t) = F.C_a(t)^* \exp(-[F/V_d + 1].t + V_a.C_a(t) \qquad (3)$$

where $C_{tot}(t)$ is the measured tissue concentration in a region of interest and V_a the arterial spillover fraction. Due to the finite resolution of PET cameras, there are the problems of spillover and of underestimation of $C_t(t)$ (the partial volume effect), which is identical for F and V_d. This can be corrected for by introducing a tissue fraction component (Iida et al, 1988):

$$F = \alpha.MBF \qquad (4)$$

$$V_d = \alpha.p \qquad (5)$$

where α is the fraction of exchangeable tissue (ml of tissue per ml of region of interest), p the partition coefficient of water (ml of blood per ml of tissue), and MBF the flow for the exchangeable tissue (ml of blood per ml of tissue per minute, or $ml \cdot min^{-1} \cdot ml^{-1}$).

In Equation 5, it was assumed that the volume of distribution of water for exchangeable tissue is the same as the partition coefficient of water for normal myocardial tissue. Substituting Equations 4 and 5 into Equation 3:

$$C_{tot}(t) = \alpha.MBF.C_a(t) * \exp(-[MBF/p + l]t) + V_a.C_a(t) \quad (6)$$

Using dynamic PET scanning, C_a (left atrium) and C_{tot} (myocardium) can be measured non-invasively as a function of time. Equation 6 can be fitted for an MBF, with V_a assuming a value for p. In the reported studies, a value of 0.96 $ml \cdot ml^{-1}$ was used based on previous data (Araujo et al, 1991). Of course, Equation 6 assumes that the partition coefficient (the amount of water in myocardial tissue) remains constant. Subsequent validation work has shown that as Equation 6 is less sensitive to resolution effects as the MBF obtained is independent of the limitations of spatial resolution, and thus less sensitive to the drawing of myocardial regions of interest. Thus, Equation 6 provides MBF data independent of both partial volume and spillover.

APPENDIX 2.

Myocardial Metabolism

At a cellular level in the normoxic state, oxidative phosphorylation of metabolic substrates occurs in the mitochondria to generate high-energy phosphates, namely adenosine triphosphate (ATP), to fuel cellular function and myocardial contractility. Under fasting conditions, the heart preferentially uses free fatty acids as its source of energy, using up to 10% of the whole body turnover despite receiving only 5% of cardiac output (Camici et al, 1989b). Oxidation of lipid-derived substrates accounts for up to 80% of myocardial oxygen consumption, with the majority of the remaining energy requirement provided by carbohydrates (glucose, pyruvate and lactate). However, with the increase in glucose loading in the fed state, glucose and insulin levels increase with subsequent reduction in fatty acid uptake through insulin inhibition of adipose tissue lipolysis. With greater production of energy from glucose for each unit of oxygen consumed, there is improved efficiency of oxygen (O_2) usage after feeding (5.01 $kcal \cdot l^{-1}$ of O_2 vs. 4.66 $kcal \cdot l^{-1}$ of O_2 from fatty acids) (Camici et al, 1989b).

Normal Cardiac Metabolism of Carbohydrate

At rest, normal subjects and patients with coronary artery disease have similar metabolism with all major substrates (free fatty acids, glucose, ketone bodies, pyruvate, lactate and glutamate) extracted to generate acetyl Coenzyme A (acetyl CoA) to fuel the tricarboxylic acid cycle (Appendix Figure A).

Glucose is transported into the cell by a specific carrier system which is not energy-dependent. Myocardial glucose uptake is determined by cardiac workload, insulin concentration, glucose concentration, the degree of adrenergic activation, and tissue oxygen tension (Camici et al, 1989b). After trans-sarcolemmal transport, glucose is rapidly phosphorylated into glucose-6-phosphate by the enzyme

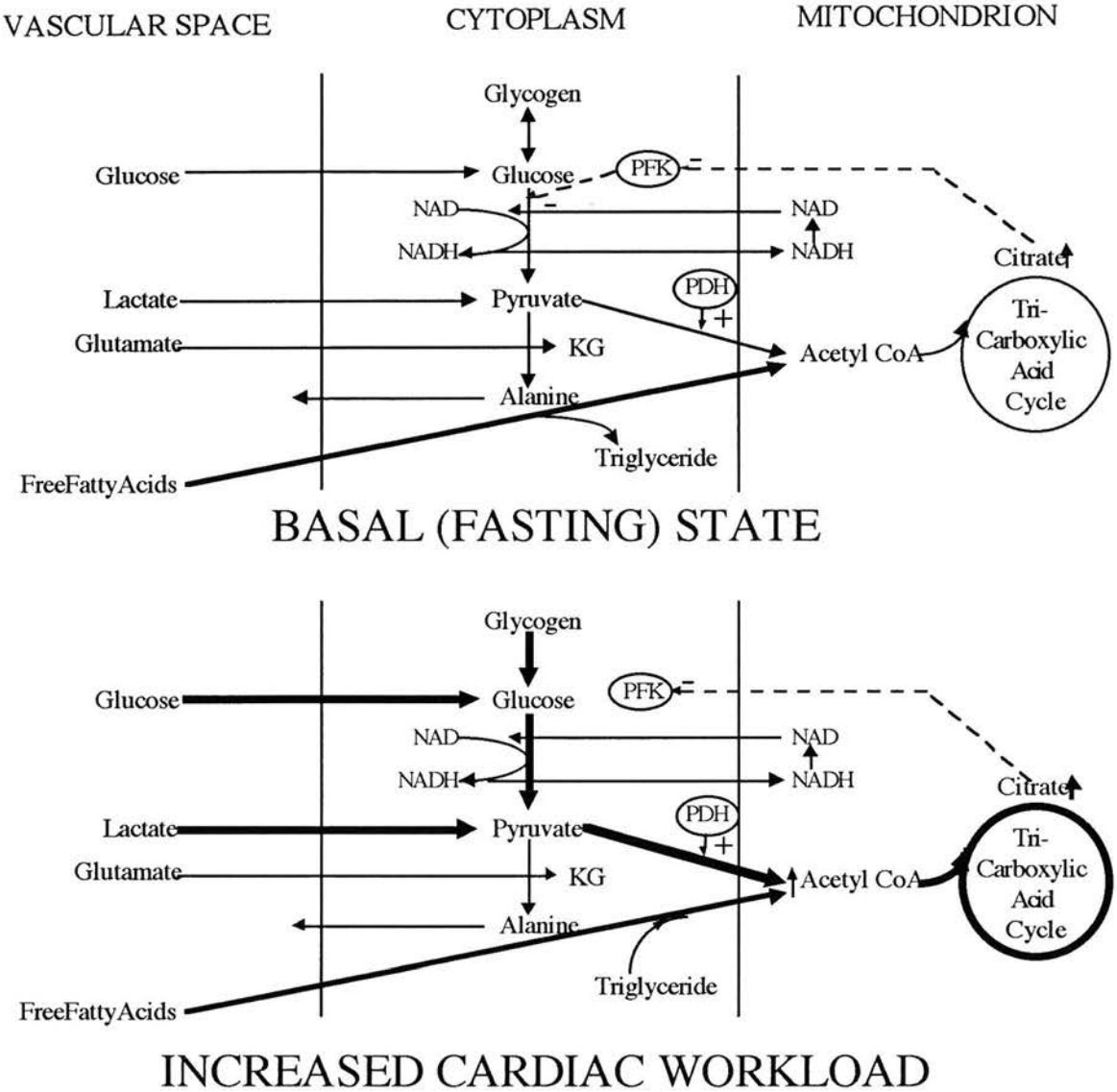


Figure A. Upper panel) The major metabolic pathways in resting human myocardium under fasting conditions. Most of the energy produced (>80%) derives from oxidation of free fatty acids. Most of the pyruvate formed from lactate and glucose through glycolysis is used aerobically and enters the tricarboxylic cycle after conversion to acetyl coenzyme A. A small amount of pyruvate is transaminated to alanine, which is then released, at the expense of glutamate which is converted to α-ketoglutarate. The inhibition of glycolysis at the level of the enzyme phosphofructokinase (PFK) is the result of accumulation of excess citrate during oxidation of free fatty acids. Pyruvate dehydrogenase (PDH) is inhibited by accumulated reduced coenzyme nicotinamide adenine dinucleotide (NADH). NADH, which is formed during glycolysis in the cytosol, is normally reoxidised (NAD) in the mitochondrion by way of the malate-aspartate cycle. **Lower panel)** Metabolic changes during increased cardiac workload in normal myocardium. There is rapid activation of glycogen breakdown and increased utilisation of exogenous glucose and lactate. Carbohydrate (glucose, lactate, pyruvate, and alanine) oxidation is significantly increased and accounts for more than 60% of energy production, acting as a booster and contributing to the extra energy required during maximum stress. (Modified from Camici et al, 1989b).

hexokinase, even in conditions of high glucose transport such as tissue hypoxia. Normally, the rate of trans-sarcolemmal transport and hexokinase activity are the limiting steps for myocardial glucose utilization. Glucose-6-phosphate may then be converted to glycogen by glycogen synthetase or to pyruvate through the glycolysis (the Embden-Meyerhof pathway). Glucose-6-phosphate may also be metabolised via the pentose-phosphate pathway but this pathway, although very important for cellular survival, is of lesser quantitative importance.

Glycolysis is controlled by feedback from the distal products of aerobic and anaerobic glucose metabolism and by fatty acid metabolism at discrete sites (Newsholme & Crabtree, 1979), which depends on whether the subject is in the fed state (because of the increased levels of circulating glucose and insulin, with increased glucose oxidation) or fasting state (where oxidation of lipid-derived substrates provides most of the energy for ATP production) (Randle et al, 1963). Hexokinase activation shifts control of glucose metabolism to the enzyme phosphofructokinase, which is a major rate-limiting step.

Phosphofructokinase activity is amplified by reduced concentrations of ATP and creatine phopshate and increased concentrations of ADP, adenosine monophosphate (AMP) and inorganic phosphate (Pi). Citrate is also a powerful inhibitor of this enzyme (Figure A). In the fasting state, fatty acid and ketone body oxidation lead to acetyl CoA production with the excess accumulation of citrate through the tricarboxylic acid cycle countering the usual positive feedback of high levels of fructose-6-phosphate (a subsequent product of glucose-6-phosphate) on phosphofructokinase, thus inhibiting glycolysis.

Pyruvate occupies a central role in the ultimate disposal of glucose: it may be reduced to lactate (completing anaerobic glycolysis); transaminated to alanine at the expense of glutamate which serves as amine group donor; or oxidised to acetyl CoA inside the mitochondrion by pyruvate dehydrogenase, which is the predominant route

under aerobic conditions. A decreased ratio of [ATP]/[ADP], the fasting state, adrenaline, and increased cardiac workload all facilitate conversion of the enzyme from the inactive to the active form. Under aerobic conditions, there is also a net balance in favour of lactate extraction from the circulation into myocardium with conversion to pyruvate, underlining the versatility of the heart to use a variety of different substrates for energy production depending on their plasma concentration. In general, in both normal subjects and patients with coronary artery disease at rest under fasting conditions, carbohydrate (mainly glucose) uptake exceeds oxidation, indicating non-oxidative disposal of these substrates.

The tricarboxylic (citric) acid cycle is tightly controlled in the mitochondria by extramitochondrial [ATP]/[ADP], intramitochondrial [NAD]/[NADH], and oxygen tension. This prevents unnecessary utilisation of substrate when ATP levels are adequate for cellular respiration. However, under conditions of stress such as exercise or atrial pacing, the heart needs to increase oxygen consumption and generate an increased amount of high energy phosphates. This is achieved by increasing utilisation of exogenous substrates such as glucose and free fatty acids whose delivery is facilitated by the increase in coronary blood flow. At moderate workload, free fatty acids remain the preferential substrate providing acetyl CoA for oxidative phosphorylation. With increasing cardiac workload, glucose uptake and utilisation increases with no further change in free fatty acid uptake (Appendix Figure A), with additional substrate for glycolysis derived from glycogen breakdown (Camici et al, 1989b). This may be because of the greater energy yield (amount of ADP phosphorylated per oxygen consumed) with glucose compared to free fatty acids.

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